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CONTENTS OF VOL. LXXVII.

No. 1. December 19, 1932.

	PAGE
Double vagotomy in relation to respiration. By G. V. ANREP and ADLI SAMAAH	1
Observations on panting. By G. V. ANREP and M. HAMMOUDA	16
The pressure of aqueous vapour in the alveolar air. By RONALD V. CHRISTIE and ALFRED L. LOOMIS	35
Studies in muscular activity. VII. Factors limiting the capacity for work. By D. B. DILL, H. T. EDWARDS and J. H. TALBOTT	49
Physiological leucocytosis. II. Post-prandial leucocytosis and Widal's hæmoclastic test for hepatic efficiency. By HAROLD ERIC MARTIN	63
The action of adrenaline upon the cardiac vagus centres. By G. STELLA	68
Phlorrhizin diabetes in fasting or fed hypophysectomized dogs. By A. BIASOTTI and B. A. HOUSSAY	81
Ketosis in the pancreatic and phlorrhizin diabetes of hypophysectomized dogs. By CIRO T. RIETTI	92
The blood cholesterol in anæsthesia. By A. C. GHOSE	97
The metabolism of lactose. Part II. The blood sugar during lactation. By L. B. WINTER	100
A negative phase in the heat production of muscle. By E. VETREE	104

No. 2. January 16, 1933.

The heat production of crustacean nerve. By MARY BERESINA and TENG	111
Localization of the action of drugs on the pulmonary vessels in dogs and cats. By J. H. GADDUM and P. HOLTZ	139
Studies on the physiology of reproduction. V. The adrenal cortex in pregnancy and lactation. By DOROTHY H. ANDERSEN and HELEN S. KENNEDY	159

	PAGE
The differential pharmacology of the inferior vena cava of some mammals. By K. J. FRANKLIN	174
The measurement of red cell volume. IV. Alterations in cell volume in hypotonic plasma. By JOHN MACLEOD and ERIC PONDER	181
The investigation of the toxic effects of large amounts of sugar in the blood. By COURTENAY EVANS	189
The rate of blood flow and gaseous metabolism of the uterus during pregnancy. By J. BARCROFT, W. HERKEL and S. HILL	194

No. 3. February 8, 1933.

The components of the retinal action potential in mammals and their relation to the discharge in the optic nerve. By RAGNAR GRANT	207
Experiments on the nature of the labile rôle of sulphur in metabolism. By H. ELLIS C. WILSON	240
The antagonistic effect of alcohol on pituitrin hyperglycæmia. By M. M. MURRAY	247
Observations on sensation. The sensory functions of the skin for touch and pain. By DAVID WATERSTON	251
Single-shock excitation and inhibition of contralateral extension in the spinal cat. By K. MATTHES and T. C. RUCH	258
Respiration of isolated gill tissue of the eel. By J. B. BATEMAN and ANCEL KEYS	271
The mucus factor in the automatic regulation of the acidity of the gastric contents. By CHARLES BOLTON and GORDON W. GOODHART	287
The histamine-histaminase system in the isolated perfused kidney-lung preparation. By R. G. MACGREGOR and S. PEAT	310

No. 4. March 15, 1933.

The central and the reflex mechanism of panting. By M. HAMMOUDA	319
Lapicque's theory of curarization. By W. A. H. RUSHTON	337

CONTENTS.

v

	PAGE
The effect of insulin on the respiratory quotient, oxygen consumption, sugar utilization, and glycogen synthesis in the normal mammalian heart in hyper- and hypoglycæmia. By E. W. H. CRUICKSHANK and C. W. STARTUP	365
The excretion of protein by the mammalian kidney. By L. E. BAYLISS, PHYLLIS M. TOOKEY KERRIDGE and DOROTHY S. RUSSELL	386
The portal circulation. II. The action of acetylcholine. By JOHN MCMICHAEL	399
The nervous control of the caudal region of the large bowel in the cat. By R. C. GARRY	422
The influence of oxygen pressure on the metabolism of the isolated cold-blooded heart. By A. J. CLARK, R. GADDIE and C. P. STEWART	432
The gastric fibres of the vagus nerve. By B. A. MCSWINEY and W. R. SPURRELL	447

LIST OF AUTHORS.

	PAGE
ANDERSEN, D. H. and KENNEDY, H. S. Adrenal in pregnancy and lactation	159
ANREP, G. V. and HAMMOUDA, M. Observations on panting . . .	16
ANREP, G. V. and SAMAAH, A. Double vagotomy and respiration .	1
BARCROFT, J., HERKEL, W. and HILL, S. Metabolism of pregnant uterus	194
BATEMAN, J. B. and KEYS, A. Respiration of isolated gill tissue of eel	271
BAYLISS, L. E., KERRIDGE, P. M. T. and RUSSELL, D. S. Excretion of protein	387
BERESINA, M. and FENG, T. P. Heat production of crustacean nerve	111
BIASOTTI, A. and HOUSSAY, B. A. Phlorrhizin diabetes and the hypophysis	81
BOLTON, C. and GOODHART, G. W. Regulation of gastric acidity .	287
CHRISTIE, R. V. and LOOMIS, A. L. Pressure of aqueous vapour in alveolar air	35
CLARK, A. J., GADDIE, R. and STEWART, C. P. Oxygen pressure and heart metabolism	432
CRUTCHSHANK, E. W. H. and STARTUP, C. W. Insulin in heart metabolism	365
DILL, D. B., EDWARDS, H. T. and TALBOTT, J. H. Studies in muscular activity	49
EDWARDS, H. T., TALBOTT, J. H. and DILL, D. B. Studies in muscular activity	49
EVANS, C. Effects of large injections of sugar	189
FENG, T. P. and BERESINA, M. Heat production of crustacean nerve	111
FRANKLIN, K. J. Pharmacology of inferior vena cava	174
GADDIE, R., STEWART, C. P. and CLARK, A. J. Oxygen pressure and heart metabolism	432
GADDUM, J. H. and HOLTZ, P. Vaso-motor changes in lungs . .	139
GARRY, R. C. Nervous control of large bowel	422
GHOSE, A. C. Blood cholesterol in anæsthesia	97
GOODHART, G. W. and BOLTON, C. Regulation of gastric acidity .	287
GRANIT, R. Components of retinal action potential	207
HAMMOUDA, M. and ANREP, G. V. Observations on panting . . .	16
HAMMOUDA, M. Central and reflex mechanism of panting . . .	319
HARTREE, W. Negative phase in muscle heat	104
HERKEL, W., HILL, S. and BARCROFT, J. Metabolism of pregnant uterus	194

	PAGE
HILL, S., BARCROFT, J. and HERKEL, W. Metabolism of pregnant uterus	194
HOLTZ, P. and GADDUM, J. H. Vaso-motor changes in lungs	139
HOUSSAY, B. A. and BIASOTTI, A. Phlorrhizin diabetes and the hypophysis	81
KENNEDY, H. S. and ANDERSEN, D. H. Adrenal in pregnancy and lactation	159
KERRIDGE, P. M. T., RUSSELL, D. S. and BAYLISS, L. E. Excretion of protein	387
KEYS, A. and BATEMAN, J. B. Respiration of isolated gill tissue of eel	271
LOOMIS, A. L. and CHRISTIE, R. V. Pressure of aqueous vapour in alveolar air	35
MACGREGOR, R. G. and PEAT, S. Histaminase and histamine	310
MACLEOD, J. and PONDER, E. Measurement of red cell volume	181
McMICHAEL, J. The portal circulation	399
McSWINEY, B. A. and SPURRELL, W. R. The gastric fibres of the vagus nerve	447
MARTIN, H. E. Physiological leucocytosis	63
MATTHES, K. and RUCH, T. C. Single-shock spinal extensor reflex	258
MURRAY, M. M. Alcohol and pituitrin hyperglycæmia	247
PEAT, S. and MACGREGOR, R. G. Histaminase and histamine	310
PONDER, E. and MACLEOD, J. Measurement of red cell volume	181
RIETTI, C. T. Ketosis after hypophysectomy	92
RUCH, T. C. and MATTHES, K. Single-shock spinal extensor reflex	258
RUSHTON, W. A. H. Lericq's theory of curarization	337
RUSSELL, D. S., BAYLISS, L. E. and KERRIDGE, P. M. T. Excretion of protein	387
SAMAAN, A. and ANREP, G. V. Double vagotomy and respiration	1
SPURRELL, W. R. and McSWINEY, B. A. The gastric fibres of the vagus nerve	447
STARTUP, C. W. and CRICKSHANK, E. W. H. Insulin in heart metabolism	365
STELLA, G. Adrenaline on cardio-inhibitory centres	68
STEWART, C. P., CLARK, A. J. and GADDIE, R. Oxygen pressure and heart metabolism	432
TALBOTT, J. H., DILL, D. B. and EDWARDS, H. T. Studies in muscular activity	49
WATERSTON, D. Observations on sensation	251
WILSON, H. E. C. Sulphur metabolism	240
WINTER, L. B. Lactose in urine	100

PROCEEDINGS OF THE PHYSIOLOGICAL SOCIETY.

October 22, 1932.

	PAGE
<i>Adams, T. W. and Poulton, E. P.</i> The fallacies of indirect calorimetry	1 P
<i>Bain, W. A.</i> On the mode of action of vaso-motor nerves	3 P
<i>Marrian, G. F. and Newton, W. H.</i> The action of œstrin on the isolated uterus	4 P
<i>Campbell, J. Argyll.</i> Gas tensions in the gall bladder	6 P
<i>Campbell, J. Argyll.</i> Hypertrophy of the heart in acclimatization to chronic carbon monoxide poisoning	8 P
<i>Daly, I. de Burgh and Thorpe, W. V.</i> An isolated mammalian heart preparation capable of performing work for prolonged periods.	10 P
<i>Fraser-Harris, D. F.</i> A simple method of appreciating the muscular sense	11 P

November 19, 1932.

<i>Hemingway, A.</i> The rate of glomerular filtration in the perfused kidney	13 P
<i>Hemingway, A.</i> A new perfusion pump	14 P
<i>Hemingway, A. and Sims, A. L.</i> A one screw-fixing boss head.	15 P
<i>Gage, F. H. and Shaxby, J. H.</i> Modification of speech sounds in passing through acoustic filters	16 P
<i>Brown, T. Graham.</i> A new differential æsthesiometer	16 P
<i>Gage, F. H. and Shaxby, J. H.</i> The regions of greatest energy in the production of vowel sounds	18 P
<i>Cooper, Sybil and Sherrington, C. S.</i> Degeneration of peripheral nerves after spinal transection in the monkey	18 P

December 17, 1932.

<i>McDowall, R. J. S.</i> A convenient method of studying the reactions of the vaso-motor centre	21 P
<i>Maizels, Montague.</i> The rate of diffusion into erythrocytes	22 P
<i>Eccles, J. C., Granit, R. and Young, J. Z.</i> Impulses in the giant nerve fibres of earthworms	23 P
<i>Fromherz, H. and Hill, A. V.</i> The action of veratrine on frog's nerve	25 P
<i>Robinson, M. H. B. and Thompson, J. H.</i> The effect on the body temperature of animals of injections of an extract of parathyroid glands	26 P
<i>Cowan, S. L.</i> The effect of veratrine on the electrical response of crab's nerve	27 P
<i>Matthews, Bryan H. C.</i> Loss of heat at high altitudes	28 P
<i>Funk, Casimir and Zeffrow, P. P.</i> Decreased urinary flow in rats on high fat diet	29 P
<i>Bhatia, B. B. and Burn, J. H.</i> Action of ether on the sympathetic system	30 P
<i>Osborne, W. A.</i> Electric symmetry of the thorax	31 P

CONTENTS.

ix

January 21, 1933.

	PAGE
<i>MacGregor, R. G. and Thorpe, W. V.</i> The extraction of histamine from tissues by electro dialysis	33 P
<i>Bayliss, L. E. and Ogden, E.</i> "Vaso-tonins" and the pump-oxygenator-kidney preparation	34 P
<i>Daly, I. de Burgh.</i> A seven horse-power Austin engine adapted as a blood pump	36 P
<i>Reid, Charles.</i> Survival after adrenalectomy in rabbits	37 P
<i>Hartridge, H.</i> The future of the cathode-ray tube in physiology	39 P
<i>Needham, Joseph.</i> The avoidance of ketosis in the cleidoic egg	41 P

LIST OF AUTHORS.

ADAMS, T. W. and POULTON, E. P. Fallacies of indirect calorimetry	1 P
BAIN, W. A. Action of vaso-motor nerves	3 P
BAYLISS, L. E. and OGDEN, E. "Vaso-tonins" and kidney	34 P
BHATIA, B. B. and BURN, J. H. Action of ether on sympathetics	30 P
BROWN, T. GRAHAM. Differential æsthesiometer	16 P
BURN, J. H. and BHATIA, B. B. Action of ether on sympathetics	30 P
CAMPBELL, J. ARGYLL. Gas tension in gall bladder	6 P
CAMPBELL, J. ARGYLL. Hypertrophy of the heart from CO ₂ poisoning	8 P
COOPER, SYBIL and SHERRINGTON, C. S. Nerve degeneration after spinal transection	18 P
COWAN, S. L. Veratrine on crab's nerve	27 P
DALY, I. DE BURGH. Large blood pump	36 P
DALY, I. DE BURGH and THORPE, W. V. Isolated mammalian heart	10 P
ECCLES, J. C., GRANIT, R. and YOUNG, J. Z. Nerve fibres of earth-worms	23 P
FRASER-HARRIS, D. F. Muscular sense	11 P
FROMHERZ, H. and HILL, A. V. Action of veratrine on nerve	25 P
FUNK, CASIMIR and ZEFIROW, P. P. Urinary flow on fat diet in rats	29 P
GAGE, F. H. and SHANBY, J. H. Distortion of speech by filters	16 P
GAGE, F. H. and SHANBY, J. H. Energy in vowel sounds	18 P
GRANIT, R., YOUNG, J. Z. and ECCLES, J. C. Nerve fibres of earth-worms	23 P
HARTRIDGE, H. Cathode-ray tubes	39 P
HEMINGWAY, A. Rate of glomerular filtration	13 P
HEMINGWAY, A. Perfusion pump	14 P
HEMINGWAY, A. and SIMS, A. L. Single screw boss head	15 P
HILL, A. V. and FROMHERZ, H. Action of veratrine on nerve :	25 P
MCDOWALL, R. J. S. Method of studying vaso-motor centre	21 P
MACGREGOR, R. G. and THORPE, W. V. Histamine extraction	33 P
MAIZELS, MONTAGUE. Diffusion into erythrocytes	22 P
MARRIAN, G. F. and NEWTON, W. H. Action of cestrin on uterus	4 P
MATTHEWS, BRYAN H. C. Loss of heat at high altitudes	28 P

	PAGE
NEEDHAM, JOSEPH. Avoidance of ketosis in cleidoic egg	41 <i>P</i>
NEWTON, W. H. and MARRIAN, G. F. Action of oestrin on uterus	4 <i>P</i>
OGDEN, E. and BAYLISS, L. E. "Vaso-tonins" and kidney	34 <i>P</i>
OSBORNE, W. A. Electric symmetry of the thorax	31 <i>P</i>
POULTON, E. P. and ADAMS, T. W. Fallacies of indirect calorimetry	1 <i>P</i>
REID, CHARLES. Survival after adrenalectomy in rabbits	37 <i>P</i>
ROBINSON, M. H. B. and THOMPSON, J. H. Body temperature and parathyroid extract	26 <i>P</i>
SHAXBY, J. H. and GAGE, F. H. Distortion of speech by filters	16 <i>P</i>
SHAXBY, J. H. and GAGE, F. H. Energy in vowel sounds	18 <i>P</i>
SHERRINGTON, C. S. and COOPER, SYBIL. Nerve degeneration after spinal transection	18 <i>P</i>
SIMS, A. L. and HEMINGWAY, A. Single screw boss head	15 <i>P</i>
THOMPSON, J. H. and ROBINSON, M. H. B. Body temperature and parathyroid extract	26 <i>P</i>
THORPE, W. V. and DALY, I. DE BURGH. Isolated mammalian heart	10 <i>P</i>
THORPE, W. V. and MACGREGOR, R. G. Histamine extraction	33 <i>P</i>
YOUNG, J. Z., ECCLES, J. C. and GRANIT, R. Nerve fibres of earth- worms	23 <i>P</i>
ZEFIROW, P. P. and FUNK, CASIMIR. Urinary flow on fat diet in rats	29 <i>P</i>

DOUBLE VAGOTOMY IN RELATION TO RESPIRATION.

BY G. V. ANREP AND ADLI SAMAAH.

(From the Physiological Laboratory, University of Cairo.)

PAVLOV [1895, 1896] and his pupils, Cachcovsky [1899] and Cheshcov [1902], succeeded in keeping dogs alive after simultaneous double vagotomy in the neck. This was the first case reported in the literature of a prolonged survival of dogs operated on in this way. One of these animals survived in Pavlov's laboratory for 19 months, and died from an accidental cause. Pavlov ascribes the success of his experiments to the fact that œsophagostomy and gastrostomy were established in all his dogs before their vagi were cut. The œsophagostomy safeguarded the animals against the penetration of regurgitated food into the respiratory passages. The diet of the animals had to be carefully selected, and the stomach had to be washed out at regular intervals through the gastric fistula, in order to prevent auto-intoxication due to the stagnation of semi-digested food in the stomach. Pavlov and his co-workers give a very detailed description of the changes in the respiratory activity of these vagotomized dogs. In all the animals, immediately after the section of both cervical vagi, the rate of respiration fell to 4-8 per minute. However, this slowing down of breathing gradually disappeared and, towards the second or third week after the operation, the respiratory rate returned almost to normal. After this the rate gradually diminished again and finally settled at 4-8 per minute, the respiration remaining at this slow rate up to the death of the animals. The immediate slowing down of the breathing, its temporary acceleration and then the final slowing down were typical of all operated animals. The respiratory slowing down is ascribed by Pavlov to the section of the sensory pulmonary fibres of the pneumogastric nerves. The temporary acceleration on the second to the fourth week after the operation is tentatively explained as being due to excitations arising in the central ends of the cut vagi during the process of healing at the place of section. Thus Pavlov's observations on the slow breathing of surviving vagotomized animals correspond with the results of all the previous authors in vivisection experiments. In

explaining this phenomenon Pavlov is also in agreement with the older observers.

This classical explanation of the slow breathing of vagotomized animals has lately been subjected to considerable criticism. Boothby and Shamoff [1915] claim to have performed a complete intra-thoracic denervation of the lungs by cutting the nerve filaments at the root of each lung while the trunks of the vagi were left intact. The animals survived for several months and showed no change either in the rate or in the depth of respiration. Moreover Boothby and Berry [1915] noticed that, in the same dogs, distension of the lungs by positive pressure produced the usual cessation of respiration (Hering-Breuer's reflex [1868]), an effect which invariably disappears after section of the cervical vagi. Thus these animals present a striking contrast to those which had their vagi cut in the neck.

Sharpey-Schafer [1919, 1932], working chiefly on cats, expressed the opinion that the slowing down and deepening of the respiratory movements in cervical vagotomy is not due to the section of the sensory pulmonary fibres, but to an increased mechanical resistance caused by the bilateral paralysis of the laryngeal muscles and the falling together of the thyro-arytenoid ligaments, the laryngeal paralysis obviously resulting from the section of the inferior (recurrent) laryngeal fibres. As a result of a few experiments performed on dogs and rabbits, Sharpey-Schafer ascribes the respiratory slowing down in these animals to the same cause.

J. F. and C. Heymans [1928], working on dogs, also deny that the respiratory slowing down is due to the denervation of the lungs. They used the same technique as Head [1889 *a, b*], and fundamentally their experiments are very similar to those of Moore [1927]. They cut one vagus in the neck and kept the animal alive by rhythmic insufflation of the denervated lung. The other, innervated, lung was collapsed. Under these conditions it was noticed that, on cutting the cervical vagus, *i.e.* the vagus on the side of the collapsed lung, the respiration assumed the typical vagal rate. In interpreting these experiments the authors assume that, in the case of denervation of one lung and collapse of the other innervated lung, all pulmonary influences upon the respiratory rate are abolished, and therefore the slow vagal type of breathing cannot be explained by a destruction of the pulmonary innervation. They regard the respiratory slowing down as due to the removal of cardio-aortic impulses, and conclude that what they call the "respiratory vagal tone" is of cardio-aortic and not of pulmonary origin.

The following are the various theories which have been advanced up to the present in order to explain the cause of the respiratory slowing down after double cervical vagotomy:

- (1) The removal of the pulmonary control of the respiratory rate (the generally accepted view).
- (2) Any other cause but the denervation of the lungs (Boothby and Shamoff).
- (3) An increased resistance in the respiratory passages due to the paralysis of the laryngeal muscles (Sharpey-Schafer).
- (4) The removal of a cardio-aortic control of the respiratory rate (J. F. and C. Heymans).

The experiments described in the present communication were performed in order to test these different theories. The observations deal with the respiratory changes which develop immediately after vagotomy, as well as with changes observed within a few days (up to the ninth day) after the operation. Adult dogs and cats were used for the experiments. The following anaesthetics were employed: chloroform and ether, chloralose (0.05–0.075 g. per kg. intravenously), sodium luminal (0.05 g. per kg. intravenously) and urethane (0.30 g. per kg. subcutaneously). A small dose of morphine hydrochloride (0.003–0.005 g. per kg. subcutaneously) was injected in some experiments before anaesthesia. All animals which were allowed to recover from the anaesthesia were operated upon aseptically.

THE LARYNGEAL THEORY OF THE RESPIRATORY SLOWING DOWN.

As a result of a great number of experiments performed chiefly on cats, Sharpey-Schafer, using various indirect methods, has come to the conclusion that the slow respiratory rhythm which develops after double cervical vagotomy is due to the paralysis of the laryngeal muscles, and not to the denervation of the lungs as was hitherto supposed. According to this observer, section of the vagi in a tracheotomized cat or dog, or after cauterization of the thyro-arytenoid ligaments, fails to produce any permanent change in the respiratory rate. Without this precaution, an obstruction to inspiration is caused by the falling together of the thyro-arytenoid ligaments, and this increased resistance is the cause of the slow vagal type of respiration. This conclusion could easily have been tested by a section of the inferior (recurrent) laryngeal nerves while the other vagal nerves are left intact. This direct test, however, was not performed. The following experiments were carried out in order to test Sharpey-Schafer's conclusion.

Both inferior laryngeal nerves were exposed in the neck, and the respiratory movements were recorded either by a stethograph placed round the lower part of the thorax or by Marey's tambour fixed to the larynx or to the chest wall. In some experiments the larynx was kept intact and in others it was dissected, separated from its pharyngeal connections, and exposed so that the movements of the thyro-arytenoid ligaments could be observed. Section of one or both inferior laryngeal nerves had no effect whatever on the depth or rate of the respiratory movements. The complete paralysis of the laryngeal muscles was verified in every experiment by direct observation. These experiments were repeated on many occasions on dogs and cats, and no difference was noticed in the result. In several dogs a length of 1 or 2 cm. of each inferior laryngeal nerve was excised in the neck under aseptic conditions; these animals were kept alive for several months. Except for the usual change in the voice, no other disturbances were apparent, and in no case was the rate of respiration changed.

In another series of experiments a cannula was tied directly into the trachea, or a wide rubber tube was passed from the mouth downwards through the rima glottidis, in order to prevent the vocal cords from possibly causing an obstruction to the movements of air in the respiratory passages. The respiratory movements were recorded as before. The inferior laryngeal and the vago-sympathetic nerves were exposed on both sides of the neck. It was noticed that, while the section of the recurrent laryngeal nerves had not the slightest effect on the rate of respiration, the section of the vago-sympathetic nerves immediately produced the characteristic slowing down. The slow rate of breathing remained unchanged whether the rubber tube inserted through the glottis was left in position or removed (Fig. 1 *A* and *B*).

If the straight limb of a T-piece tube is introduced into the trachea, the animal can be made to breathe either freely through the open limb of the T-piece or through the larynx. A vagotomized animal does not change its rate of respiration in either case (Fig. 1 *C*).

Sharpey-Schafer believes that the slow rate of breathing in vagotomized animals is due to an inspiratory collapse of the thyro-arytenoid ligaments. It is, however, easy to show that, while an increased resistance to expiration brings about a slowing down of breathing [Davis, Haldane and Priestley, 1919], an increased resistance to inspiration leaves the respiratory rate almost unchanged. For instance in one experiment the trachea of the dog was attached to two Boullitte respiratory valves, each of which was in turn connected with a Müller's water valve. By

lowering or raising the tube in the inlet or outlet Müller's valves, the inspiratory or the expiratory resistance respectively could be changed. The animal was breathing normally at the rate of 23-26 per minute. On increasing the inspiratory resistance to 11 cm. of water, the respiratory rate remained almost unchanged, while even a small increase of the

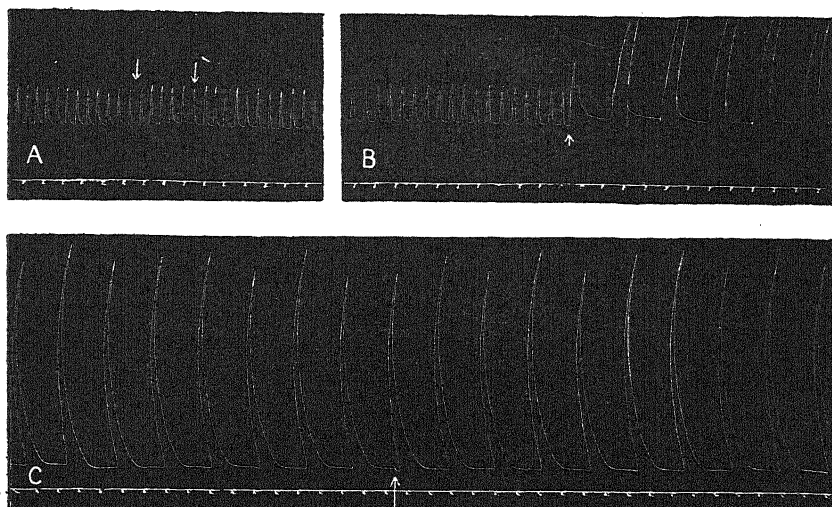


Fig. 1. Dog 7 kg. Chloralose. The respiratory movements of the chest are recorded by a stethograph connected to a Marey's tambour. Inspiration upwards. *A*. The section of the right and then the left inferior laryngeal nerve is indicated by arrows. The respiratory rate (24 per minute) was unaffected by the section. It remained constant for 90 min. when the right vagus was cut in the neck. A few minutes after the section the respiration assumed the rate of 20 per minute and remained constant for another 90 min. at which point (at the arrow in *B*) the left vagus was cut in the neck. The respiration immediately slowed down to 5-6 per minute and remained unchanged until the end of the experiment. A T-shaped cannula was introduced into the trachea which allowed the dog to breathe either through the open limb of the cannula or through the larynx. *C*. Taken 60 min. after the previous tracing. During the whole time the animal was breathing through the open limb of the cannula. At the arrow it was made to breathe through its larynx. The respiratory rate remained unchanged. This and the following tracings should be read from left to right. Time in all tracings in 5 sec.

expiratory resistance to 4 cm. of water reduced the rate from 26 to 16 per minute. This effect of increased resistance to expiration is, however, noticed only when the vagi are left intact. After section of the cervical vagi, an increase in the resistance fails to produce any effect on the rate of the respiration, unless the resistance is so great that it produces

asphyxiation of the animal. Since the increase in the expiratory resistance is effective only in the presence of the vagi, it cannot be regarded as a cause of the slow respiration in absence of the vagi.

The experiments described above fail to support Sharpey-Schafer's explanation. In adult dogs the paralysis of the laryngeal muscles cannot be considered as the cause of the respiratory slowing down following double cervical vagotomy.

THE CARDIO-AORTIC THEORY OF THE RESPIRATORY SLOWING DOWN.

J. F. and C. Heymans consider that the "respiratory tone" of the vagus nerve is of cardio-aortic origin, and that the slow vagal type of respiration is due to section of the cardio-aortic nerve fibres. No direct evidence in support of this view is, however, provided. They base their theory on the observation that section of the cervical vagi during a deflated condition of the lungs leads to a slowing down of the respiration, similar to that when the vagi are cut during normal movements of the lungs and of the respiratory muscles. But since deflation of the lungs normally produces a considerable acceleration of the respiratory movements, it would be expected that section of the vagi would lead to a comparatively greater slowing down when made during deflation of the lungs than when made on an animal which breathes spontaneously, or whose lungs are rhythmically insufflated. An inflation of the lungs produces the well-known Hering-Breuer's arrest or considerable slowing down of the respiratory movements. The extent of the effect of double vagotomy is at least in part dependent on the degree to which the inflations of the lungs have been inhibiting the respiration before the section.

The theory of the alleged cardio-aortic origin of the "respiratory vagal tone" could be directly tested by observing the effect on respiration of a complete destruction of the cardio-aortic innervation. Moreover, if this theory is correct, section of the cervical vagi after a cardio-aortic denervation should produce no effect on respiration. The chest of the animal was opened along the mid-sternal line and the internal mammary blood vessels were cut between ligatures. The depth and rate of the artificial ventilation was adjusted to prevent the development of apnoea, so that the animal performed respiratory movements during the whole experiment. These movements, as is well known, are synchronous with the periods of deflation of the lungs. The respiratory movements were registered by recording the movements of the larynx. The vagi above the heart were now carefully dissected, and all their branches between the

lower cervical ganglia and the roots of the lungs were cut. In some experiments the stellate ganglion and the thoracic sympathetic chain were also extirpated on both sides. The cardio-aortic innervation was thus destroyed, and the completeness of the destruction was shown by the disappearance of the vagus restraint of the heart. Moreover, it could be tested by an electric stimulation of the cervical vagus which, even when strong currents were used, produced no change whatever in the heart rate. It was found in these experiments that the section of the cardio-aortic fibres did not lead to the classical slowing down of the respiration. If, however, in these animals the main vagus trunks were cut in the neck, the slow vagal type of respiration made an immediate appearance.

It might possibly be objected that the animal was not in a normal condition because its respiratory movements were governed by the artificial ventilation of the lungs. In order to meet this objection, we modified the experiment in the following way. The right vagus was cut in the neck, the chest was opened, under artificial ventilation, through the second and third intercostal spaces on the left side (the third rib was left intact). By separating the ribs by means of retractors, the opening could be made sufficiently wide to allow a complete dissection of the left vagus, all the cardio-aortic branches of which were now severed. The completeness of the destruction of the cardio-aortic fibres was again tested by the stimulation of the intact vagus (left) in the neck; in no case was any change observed in the heart rate. After the end of the dissection, the chest wall was closed by suturing the intercostals and other thoracic muscles and the skin. The lungs were inflated in order to expel the air from the pleural cavity just before finally closing the wound. Artificial ventilation was then discontinued and the animal was kept under observation for over an hour. During the whole of this time, the respiratory movements of the animal showed no evidence of slowing down. Section of the left cervical vagus (the right nerve having been previously cut) led to an immediate development of the slow vagal type of respiration, although the cardio-aortic fibres had already been destroyed. This was always verified by dissection at the end of the experiment.

The following are the actual respiratory rates observed in one particular experiment. Before the section of the right cervical vagus, the rate of respiration was 26 per minute. After the section of the right nerve the rate slowed down to 18-20 per minute. After the section of the cardio-aortic fibres on the left side and after re-establishing the natural respiration, the respiratory movements showed some variations for a few minutes after the removal of the artificial ventilation, and then

settled down to the same rate of 18–20 per minute. This rate remained unchanged for about an hour. Cutting the left cervical vagus abruptly reduced the respiration to 10–11 per minute. The respiration remained slow and deep until the end of the experiment (over 4 hours). An actual record taken from a slightly modified experiment is reproduced in Fig. 2.

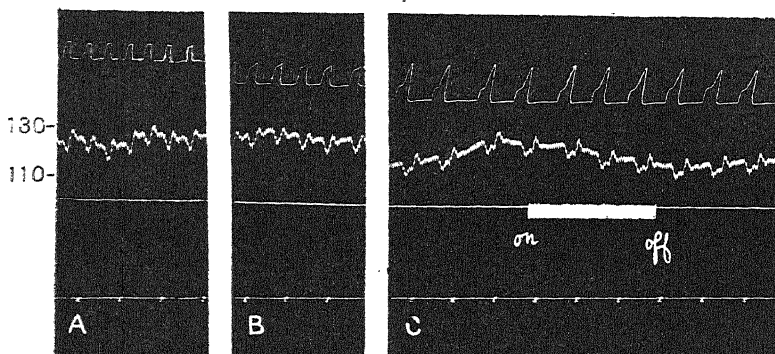


Fig. 2. Dog 10 kg. Chloralose. Stethogram and carotid blood-pressure. *A.* Record taken, 1 hour after the destruction of the cardio-aortic nerves on the left side. Respiration = 27 per minute. *B.* Record taken 45 min. after section of the right vagus in the neck; respiration = 22.5 per minute. The cardio-aortic innervation has thus been destroyed. In spite of this, the section of the left cervical vagus caused an immediate slowing of the respiration to 12–13 per minute and remained at this slow rate to the end of the experiment. *C.* Record taken 3 hours after section of the left cervical vagus. The respiratory rate = 13 per minute. The completeness of the cardio-aortic denervation is shown by the absence of the cardiac slowing during strong stimulation of the peripheral end of the cut left cervical vagus (stimulation is indicated by the signal).

The results of these experiments lead us to the conclusion that the cardio-aortic innervation cannot be responsible for the “respiratory vagal tone.” Thus our experiments do not support the view advanced by J. F. and C. Heymans¹.

¹ In the discussion which followed the communication of this research to the XIVth International Congress in Rome, Prof. C. Heymans stated that his experiments led him to accept the pulmonary origin of the respiratory vagal tone. He does not, however, consider that this tone is entirely determined by the sensory impulses arising in the lungs. In part he believes it to be due to cardio-aortic impulses. We are unable to state whether, under some special conditions, impulses may arise in the cardio-aortic region which may modify the rate of the respiration. We cannot, however, find any evidence that cardio-aortic impulses invariably exert on the rate of the respiration a regulating influence which can be compared with the definite and constant dependence of the respiratory rate on the pulmonary innervation.

THE PULMONARY THEORY OF THE RESPIRATORY SLOWING DOWN.

It is obvious that the most decisive proof for or against the pulmonary origin of the "respiratory vagal tone" would be provided by an experiment in which both lungs are completely denervated while all the other vagal fibres are left intact. This experiment was attempted by Boothby and Shamoff with a negative result. As mentioned above, they did not observe any change in the rate of respiration of their animals. Working aseptically on dogs, Boothby and Shamoff cut the branches of the vagi given off between the recurrent laryngeal nerves and the point where each vagus divides into its two primary gastro-intestinal branches. They claim that in this way all pulmonary and possibly a few cardiac branches are divided. Anyone who attempts this operation, however, will find that it is extremely difficult to make sure that all the pulmonary fibres have really been severed. It seemed to us that the denervation of the lungs could be carried out more simply and with a greater degree of certainty by the method which Anrep, Pascual and Rössler [1932] used in their experiments on cardiac arrhythmia. Thus, taking advantage of the anatomical distribution of the vagal fibres, it is possible to determine the dependence of the respiratory rate on the pulmonary innervation, by comparing the effects of section of the main trunks of the vagi just below and just above the roots of the lungs. Anrep and Segall [1926] showed that the section of the vagi just above the hila of the lungs does not disturb the cardio-aortic innervation. Indeed a careful dissection at the root of the lung shows that, besides a few œsophageal branches, all the other vagal branches in this region go to the lungs.

SECTION OF THE VAGI BELOW THE ROOTS OF THE LUNGS.

The chest wall was opened through the fifth intercostal space on the left side, the lungs being ventilated by the constant insufflation method of Meltzer and Auer. C. F. Palmer's small oscillating respiration pump was used for this purpose. The wound was widened by means of self-retaining retractors, and the branches of the vagi just below the hila of the lungs were exposed on the œsophagus. The nerves were surrounded by wires of two electro-cauteries, which were well protected so as not to touch any adjacent tissues. In some experiments sharp wire snares were used instead of electro-cauteries. The snares were arranged in such a way that they would cut through the nerves without displacing them by the traction. In experiments in which the nerves were to be stimulated,

shielded electrodes were used. The chest wall was then closed and the insufflation of the lungs was discontinued. The animals were allowed to recover for 1 or 2 hours, after which the vagi were cut by the snare or the electro-cautery. During this operation the chest remained closed.

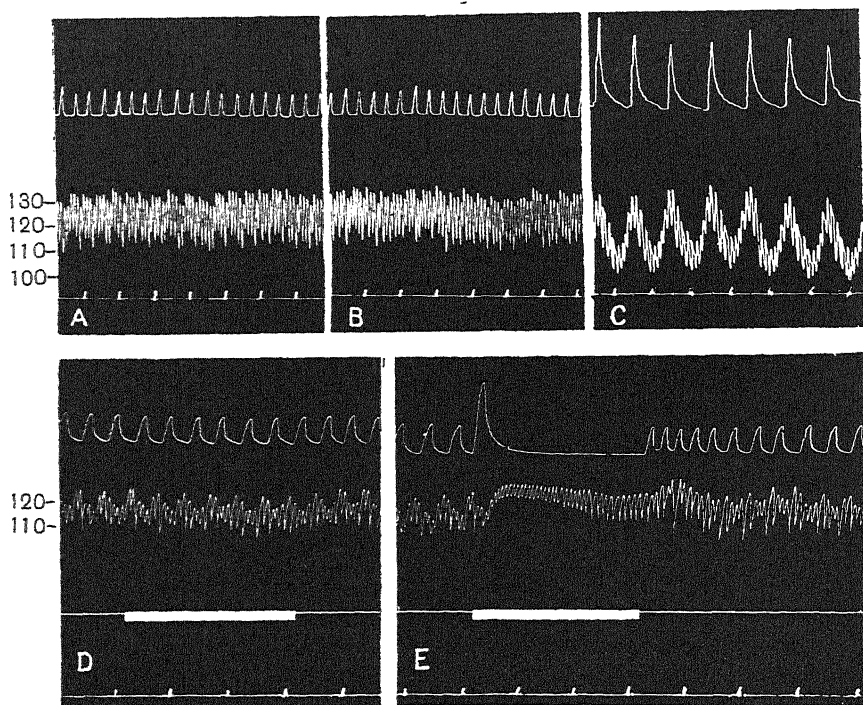


Fig. 3. Dog 6 kg. Sodium luminal. Stethogram and carotid blood pressure. *A*. Normal rate of respiration = 30 to 32 per minute. *B*. Record taken 20 min. after the section of the vagi below the roots of the lungs. Respiration = 32 per minute. *C*. Record taken 30 min. after section of the vagi just above the roots of the lungs. Respiration = 11.3 per minute. Records *D* and *E* are taken from another experiment. Dog 7 kg. Chloralose. *D*. Shows the effect on respiration of stimulation of the central end of the right cut vagus just below the root of the lung. *E*. Shows the effect on respiration of stimulation applied to the same nerve just above the root of the lung. The strength and the length of application of the stimulus were the same in both cases.

The section of the vagal trunks just below the hila of the lungs had no effect on the respiratory rate (Fig. 3 *A* and *B*). Electric excitation of the central ends of the cut nerves even with very strong currents also had no effect (Fig. 3 *D*).

In a few experiments, in order to be quite certain that all the vagal branches below the lungs were destroyed, we made a somewhat wider incision in the chest wall and, besides cutting the vagi as before, we also cut the œsophagus between the two ligatures. This, however, did not alter the result of the experiment. In every case the completeness of the section of the vagi was verified by dissection.

In three dogs the vagi were cut aseptically just below the hila of the lungs, and the animals were kept alive for 5 months. These animals showed no change in rate or depth of their respiration.

SECTION OF THE VAGI ABOVE THE ROOTS OF THE LUNG.

The same methods were used for the section of the vagi just above the roots of the lungs. On the left side the vagus was approached through the third, and on the right side through the fourth intercostal space. The electro-cautery, the snare, or the shielded electrodes were placed round each nerve well below the cardio-aortic branches, at the point where it disappears behind the hilus of the lung. Special care was taken not to include the inferior laryngeal nerve. Four animals were operated upon aseptically and kept alive for a few days. Section of the vagi just above the hila of the lungs, performed after a preliminary section just below the hila, shows the effect of pulmonary denervation.

It was found in every experiment (48 dogs) that section of the vagi above the hila produced permanent slowing down of the respiration (Fig. 3 *C*). Electric excitation of the central ends of the cut nerves, even with weak currents, causes an immediate arrest of the respiratory movements (Fig. 3 *E*).

The fate of the operated animals will be discussed in another communication. It should be mentioned, however, that during the first few days of their survival (7–9 days) the respiration retained its typical slow character (5–9 per minute). The effect of section of the vagi above the hila was observed with different forms of anæsthesia. Ether is the worst type of anæsthetic for these experiments. In ether anæsthesia the respiratory centre is unduly excited, and section of the vagi just above the hila produces a considerably smaller effect than usual. In this we confirm the observations of M'Dowall [1927] and of Trevan and Boock [1922].

In a number of experiments, instead of cutting the vagi above the roots of the lungs, we tried to make an exact reproduction of the operation of Boothby and Shamoff; that is, the pulmonary branches them-

selves were cut, while the thoracic vagal trunk was left intact. These experiments were performed as follows. The left vago-sympathetic was cut in the neck, the chest was opened and the hilus of the right lung was exposed. The number of the branches going from the vagus nerve to the lung varies in different animals; usually there are about 5-7 separate

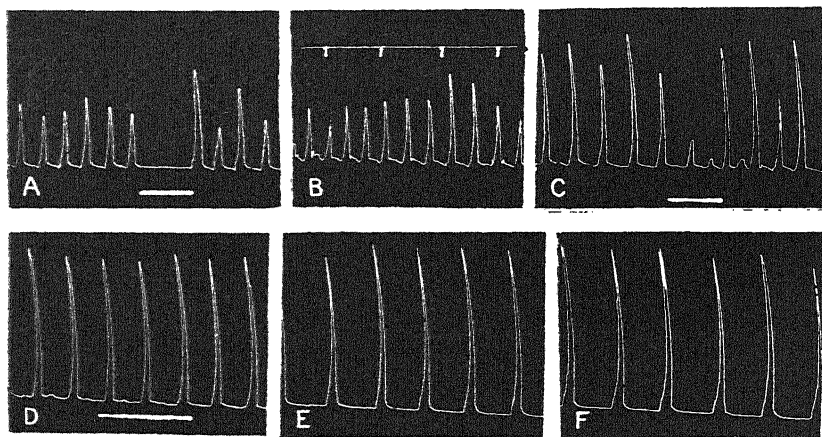


Fig. 4. Dog 6 kg. Chloralose. The respiration is recorded by registering the movements of the larynx. *A.* 40 min. after the section of the left vago-sympathetic nerve in the neck. Respiration = 32 per minute. An inflation of the lungs, indicated in the tracing by the white line, caused an arrest of the respiratory movements. *B.* 10 min. after section of the lower pulmonary branches of the right vagus at the root of the lung. Respiration = 32 to 36 per minute. The effect of inflation was unchanged. *C.* 10 min. after section of the middle pulmonary branches of the right vagus. Respiration = 23 per minute. Hering-Breuer's reflex is diminished in strength. *D.* 10 min. after section of the upper pulmonary branches. Respiration = 18.5 per minute. Hering-Breuer's reflex is absent. *E.* 15 min. after section of the smaller pulmonary filaments. Respiration = 13 to 15 per minute. *F.* The result of section of the right vago-sympathetic nerve in the neck. Respiration rate in the reproduced tracing = 13.5 per minute. The respiration actually fluctuated between 13 and 15 per minute. The rectal temperature of this animal was 39.8° C. which accounts for the slightly higher respiratory rate than that usually observed in the other experiments. The room temperature was 35.5° C.

branches. One or two big branches lie covered by the azygos vein, and can only be found after displacing the vein, or preferably after cutting it between two ligatures. Besides these branches there is a considerable number of very fine nerve filaments running from the vagus nerve to the lung. The pulmonary branches were cut in three stages starting from below upwards. After this the vagus trunk was completely separated from

the lung, to destroy the finer filaments. The completeness of the denervation of the lung was tested by cutting the vagus trunk above the pulmonary hilus. After each section the lungs were inflated in order to test the Hering-Breuer reflex. Fig. 4 shows the results obtained in one experiment. It can be noticed that the respiration begins to slow down only after at least two-thirds of the pulmonary branches were severed. The Hering-Breuer reflex at this stage is still present, but reduced in strength. After section of all the pulmonary branches, the respiration slows down still further and the Hering-Breuer reflex disappears. The lungs, however, still continue to exert an effect on the respiratory rate, as shown by the fact that the respiration shows a further slowing down after a complete separation of the vagus trunk from the lung. Section of the vagus trunk above the root of the lung, or in the neck, now completely fails to produce any change in the respiratory movements.

These experiments were repeated on animals whose chests were closed to avoid the complications introduced by the artificial ventilation of the lungs. The chest was opened on the left side, and the vagus trunk was completely separated from the hilus of the lung. After closing the chest, the animal was allowed to recover for an hour or two. Thus the laryngeal and the cardio-aortic innervation was preserved on both sides, while the pulmonary innervation was left intact on the right side only. The respiratory rate in one such experiment was 19-20 per minute. A complete denervation of the lungs was now made by cutting the right vagus in the neck. This reduced the respiratory rate to 10-11 per minute. A section of the left cervical vagus produced no further change in the respiratory rate. In other words, this section did not augment the effect of the denervation of the lungs, which would be the case if the "respiratory vagal tone" were even in part due to sensory impulses of cardio-aortic origin.

The comparison of the effect of section of the vagi below and above the roots of the lungs demonstrates the extent to which the classical changes in the respiration are produced by the denervation of the lungs. The following experiment shows that the pulmonary denervation entirely accounts for these changes, and that the destruction of other sensory fibres of the vagi does not assist in the production of the vagal type of respiration. Both nerves were cut just below the hila, electro-cauteries were placed around the vagi immediately above the hila, and the cervical vagi were exposed in the neck. As mentioned before, section of the nerves below the hila had no effect on respiration. Section immediately above the lungs reduced the rate of respiration from 24 to

7 per minute. Both cervical vagi were cut 1 hour later, without producing any further change in the respiratory rate, which remained at 7-8 per minute until the end of the experiment (see Figs. 5 *A* and *B*, which are taken from another experiment). This observation is additional evidence against the cardio-aortic theory of Heymans, as well as against the laryngeal theory of Sharpey-Schafer.

The classical slowing down of respiration is not only produced by section of the vagi, but also by blocking the nerves by means of a constant current, or by the injection of novocaine (2 p.c.) into the trunks of the nerves. The block so produced is obviously only temporary; the effect of novocaine lasts for about an hour, and depends on the concentration and

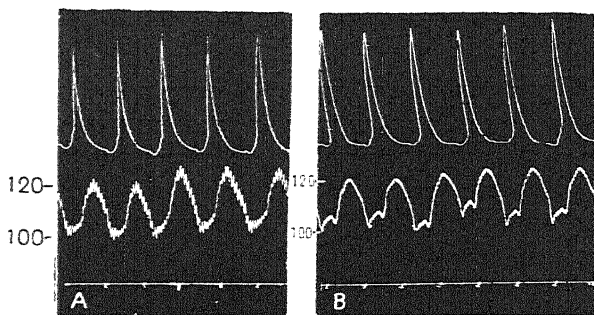


Fig. 5. Dog 7 kg. Chloralose. Stethogram and blood-pressure. *A*. One hour after the bilateral section of the vagi just above the roots of the lungs. Respiration=9 to 10 per minute. *B*. One hour after the section of both cervical vagosympathetic nerves in the neck. Respiration=9 to 10 per minute.

the amount of solution injected. Blocking the nervous impulses in the vagi immediately above the roots of the lung produces the same effect as their section. Section of the cervical vagi, after their novocainization, just above the lungs fails to produce any further slowing down.

Bilateral intrathoracic section of the pulmonary branches of the vagi, performed aseptically with the view of keeping the dog alive for a long period of time, is an operation not easy to perform. We believe that the negative results obtained by Boothby and Shamoff are due to their having missed some of the fibres. This is especially likely because Boothby and Berry obtained a positive Hering-Breuer response in these animals. The observations described here show that, if a small number of the pulmonary fibres remain intact, this reflex can still be obtained.

SUMMARY.

1. The pulmonary, laryngeal and the cardio-aortic theories of the respiratory slowing down which follows double cervical vagotomy have been subjected to an experimental test.

2. The experiments fail to support Sharpey-Schafer's view that the classical respiratory slowing down is due to the paralysis of the laryngeal muscles.

3. They also fail to support Heymans' view that it is due to a cardio-aortic denervation.

4. The effect of double vagotomy on respiration is due to the denervation of the lungs.

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OBSERVATIONS ON PANTING.

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THE first systematic investigation of the peculiar form of heat regulation by panting, observed in dogs, was made by Richet [1898]. As regards the mechanism of its action, Richet divides panting into reflex and central. Reflex panting is observed only in non-anæsthetized dogs, and is not necessarily accompanied by a rise in body temperature. When a dog is exposed to the sun or to heat, panting occurs within a few minutes, although the rectal temperature does not rise and may even drop below the pre-panting level. Anæsthesia completely abolishes the reflex type of panting. In anæsthetized dogs, panting can only be obtained by raising the body temperature. When an anæsthetized dog is exposed to heat, the respiration begins to accelerate very gradually until the rectal temperature reaches 41.7°C ., at which point the animal begins to pant. According to Richet, the reflex type of panting is due to a reflex excitation of the brain centres in response to the application of warmth to the skin and to the terminations of the fifth nerve, while the central type is due to direct stimulation by heat. Since Richet's work there has been no other systematic investigation of panting, although a good deal of research has been done on the changes which take place in the animal in response to raising the environmental temperature. In most of these researches, however, panting is discussed only as a secondary phenomenon. Bazett [1927] gives a detailed review and most of the literature on the subject.

The present investigation deals chiefly with that form of panting which Richet calls central, *i.e.* panting which is produced by raising the body temperature in anæsthetized dogs. The object of the experiments described here was to investigate the various changes taking place in the animal during panting and to study their interrelation.

TECHNIQUE.

The majority of the experiments were performed on dogs weighing from 4 to 8 kg. The animals were anaesthetized either with urethane or, after administration of a small dose of morphine (5 mg. per kg.), with chloralose. Urethane and chloralose were injected intravenously, the first in doses from 0.25 to 0.5 g. per kg., the second in doses from 0.03 to 0.07 g. per kg., according to the depth of anaesthesia desired. Cats were used in a few experiments for the purpose of comparison. They were anaesthetized with urethane. The warming of the animals was carried out in a suitably arranged box, inside which were placed several carbon filament lamps. These lamps were completely shielded with metal screens. The temperature of the air inside the box could be raised to 50° C. or more within a few minutes. By extinguishing some of the lamps, the temperature of the box could be maintained at any desired level for an indefinite length of time. The animal was placed on thick pads of cotton-wool in the grooved bottom of the box, and consequently it did not touch any hot parts. The rectal temperature was measured throughout the experiment by a sensitive mercury thermometer, which was accurate to 0.1° C. The thermometer was introduced deeply into the rectum and was observed through a small opening in the box. The head of the animal protruded from the box and was supported by a holder.

The rate of respiration was registered by means of a stethograph and recorded on smoked paper or photographically. The total ventilation was recorded by a large Krogh's spirometer of 3.0 litres capacity, into which the animal expired through an outlet Douglas' or preferably Boullitte's valve, which was directly connected with a T-shaped tracheal cannula. The animal inspired air through an inlet valve on the other side of the cannula. The tracheal cannula was inserted as low down as possible so that the total dead space would be of the same order as that of the animal under normal conditions. The oxygen consumption was recorded by making the animal breathe through the same valves into a closed system of 22 litres capacity. The outlet tube of the trachea was provided with a carbon dioxide absorber (Siebe Gorman) and a small Krogh's spirometer of 700 c.c. capacity to record the respirations. Of the 22 litres of air in the system not more than 200 c.c. were allowed to be consumed at a time, after which the oxygen was replaced. The maximum drop of the oxygen concentration in the system was thus inappreciable.

The depth of respiration (stroke) was either calculated from synchronous records of the ventilation and of the respiration rate, or it was

registered directly by means of a Brodie's bellows recorder in a closed system of two Woulfe's bottles of 10 litres capacity, connected together by wide rubber tubing. The animal inspired from one bottle and expired into the other bottle, the carbon dioxide absorber being omitted. This method has the disadvantage of not being suitable for long observations, as the oxygen is used up and the carbon dioxide accumulates in the system. For this reason the bottles had to be renewed at short intervals, and only short records were taken. On the other hand, this method has the advantage of directly registering even negligible variations of individual strokes which can only be estimated roughly by the first method.

For analysis of the blood, samples were drawn under paraffin from the carotid artery. The carbon dioxide content was determined with van Slyke's constant pressure apparatus, while the oxygen saturation was determined with Haldane's blood-gas apparatus.

THE TEMPERATURE LIMITS AND THE RATE OF PANTING.

On exposing an anæsthetized dog to heat, its rectal temperature rises slowly and its respiration accelerates more or less in relation to the rise of body temperature. At a certain temperature, however, this relation breaks and the respiration suddenly accelerates much further, although the temperature may not rise any more. Richet found that this abrupt change in the respiration took place always at 41.7°C . But the temperature of the onset of panting is found to be very dependent on the degree of anæsthesia. With light chloralose anæsthesia it develops at $39\text{--}40^{\circ}\text{C}$., with deeper anæsthesia it develops at $40.5\text{--}42^{\circ}\text{C}$., while in very deeply anæsthetized dogs panting may not develop at all, even with a rectal temperature of $42\text{--}43^{\circ}\text{C}$. An increase in the depth of anæsthesia in a panting dog abolishes panting, and it can only be re-established by a further rise of body temperature. When the animal is cooled, panting disappears if the rectal temperature falls by about 0.5°C . in deep anæsthesia, and sometimes by over 1.0°C . in light anæsthesia below the temperature of the onset of panting. Frequently, in lightly anæsthetized dogs, panting continues until the temperature is normal. The rate of panting depends on the depth of anæsthesia as well as on the temperature of the body. In light anæsthesia the usual rate of panting is 200–350 per minute, while in deep anæsthesia the rate may be as low as 120 per minute. With a further rise of temperature, the rate accelerates to the extreme which the anæsthetic can allow. It is frequently observed that in lightly anæsthetized animals the panting rate assumes periodic varia-

tions. Groups of fast and slow rates follow one another, sometimes with surprising regularity. These changes are generally, but not invariably, accompanied by changes in the stroke. The periods of fast rates may be of the same increased or diminished stroke.

We were able to confirm Richet's observation that panting is unchanged by the section of the cervical vagi. We found that the temperature limits and the maximum rate of panting are the same in vagotomized and in normal animals, if the depth of anæsthesia is controlled. It was suggested by Uyeno [1923] that, after section of the vagi, panting develops at a lower temperature and that the maximal rate obtained is lower than with the vagi intact. This may frequently be the case, but, since the onset of panting depends so much on the depth of anæsthesia, these results are inconclusive. We have on record many observations in which, at the same body temperature, panting reached a considerably higher rate after double vagotomy. If the anæsthesia is well controlled, we find no difference in the temperature of onset. Table I shows two observations on a 4.5 kg. dog which was given 0.3 g. chloralose, warmed and then cooled, with the cervical vagi intact. The vagi were then cut and the observation was repeated. During the second half of the experiment the animal was less under the effect of the anæsthetic. The rate of warming was the same in both cases.

TABLE I.

Rectal temp. °C.	Respiratory rate per min.	
	Vagi intact	Vagi cut
38.0	18	5
40.0	26	6
40.9	80-130	10-130-230
41.2	250	360
40.0	36	6
38.0	16	4

This experiment shows that, although the anæsthesia was somewhat less in the second case, panting developed at the same temperature and the rate after vagotomy was considerably higher than before. That the vagi do not play any rôle in panting is definitely shown by the fact that the respiration rate remains unchanged if the section of the vagi is made during panting (see Fig. 1).

In dogs with the vagi cut, the onset of panting is extremely abrupt. With the rise of rectal temperature, the slow and deep respirations of 4-8 per minute accelerate at first very slowly to 20-30, but when the panting temperature is reached the respiration jumps in a few seconds

to 120–150 per minute and assumes the characteristic shallowness of panting. The recovery from panting is also very abrupt, the shallow fast respirations suddenly becoming slow and deep as soon as panting disappears. The periodic variations in the rate and stroke are also observed after double vagotomy.

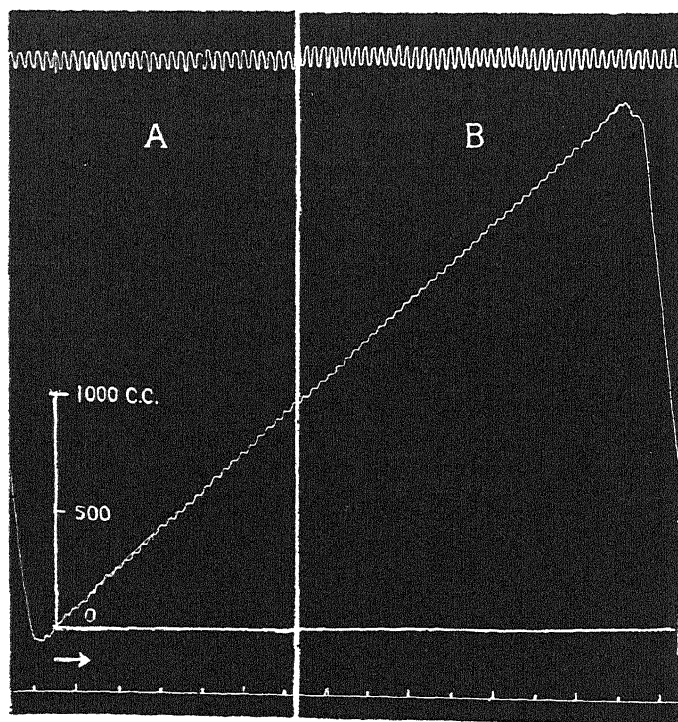


Fig. 1. The top record is the respiratory rate recorded by a stethograph, and the ascending undulating line is a record of the total ventilation. Time in seconds. Panting at 40.2° C. rectal temperature. Both cervical vagi were cut between records A and B. The interval between the two records was 30 sec. The respiratory rate was 290 and the ventilation 10,000 c.c. per minute in both records.

THE STROKE AND THE PULMONARY VENTILATION.

Contrary to the opinion of previous authors, who regarded panting as a form of dyspnoea, Richet showed that panting is "a facilitated form of shallow and fast respiration." Other workers have confirmed the observation that the panting stroke is essentially shallow. In fact, normal dogs exposed to the sun always pant with a very shallow stroke,

unless the body temperature rises to an appreciable extent. On the other hand, when panting is caused by severe exercise such as running, the panting stroke is markedly deep. In anæsthetized dogs the changes in the stroke take a characteristic course. Between subnormal and normal temperature the stroke gradually increases; between normal and the panting temperature the stroke diminishes as the rate of respiration increases, but the diminution is small. As soon as panting sets in, however, the stroke rapidly diminishes to half the original or even less. In mild panting the stroke remains shallow; it returns to its original volume only after recovery from panting. In severe panting the stroke at first also becomes shallow, as in mild panting, but it gradually increases again to the original volume and may even considerably exceed it. On recovery from severe panting the stroke retraces the same course, becoming shallow at first and then gradually increasing to the original volume. Table II gives an example of these changes in the stroke.

TABLE II.

Warming				Cooling			
Rectal temp. °C.	Rate per min.	Stroke in c.c.	Ventilation in c.c. per min.	Rectal temp. °C.	Rate per min.	Stroke in c.c.	Ventilation in c.c. per min.
37.0	25	38	960	41.0	200	67	13,300
37.5	27	42	1,130	40.5	200	60	12,000
38.0	30	43	1,290	40.0	190	54	10,200
38.5	44	37	1,630	39.5	190	37	7,000
39.0	120	25	3,000	39.0	190	28	5,400
39.5	120	28	3,360	38.5	160	28	4,500
40.0	160	45	7,200	38.0	100	26	2,600
40.5	180	81	14,600	37.5	30	40	1,200
41.0	190	99	18,800	37.0	18	45	820
41.5	210	86	18,060				

The increase in stroke observed in severe panting is due to the rise of the animal's temperature after panting has already been established. If, after the onset of panting, the body temperature is not raised any further but kept strictly constant, the stroke remains small. Usually, however, in prolonged panting in anæsthetized animals, the stroke increases as panting continues. In this respect it is difficult to imitate the continued shallow type of panting obtained in dogs which, when exposed to the sun, pant at a normal body temperature, keeping up a fast rate of 300 per minute or more without increasing the stroke.

The ventilation during mild panting, in spite of the shallow stroke, increases 6-7 times above normal with rates of 120-150 per minute. In severe panting which is accompanied by an increase in stroke, the ventilation may increase 10-26 times with rates of 200-350. Frequently

it can be noticed, especially in severe panting, that groups of large and small strokes alternate with or without changes in rate. The most frequent case is a combination of fast rates with large strokes alternating with slower rates with small strokes, the ventilation obviously following the product of the change in stroke and rate. Section of the vagi in the neck performed during panting does not change the depth of the respiration. Since the respiratory panting rate is not affected by double vagotomy, the total ventilation obviously also remains unchanged (see Fig. 1).

In similar experiments on cats under urethane, Uyeno [1923], working in Cambridge, found that the highest rate of panting did not exceed 250 per minute and that "this change is not associated with a corresponding change in the total ventilation per minute which so far from being increased twelve or fifteenfold was never increased threefold, and on one occasion was not increased at all." In consequence of this, the respiration became very shallow, the observed respiratory depth being of the order of 2-3 c.c. or even less. As a result of this very shallow respiration the oxygen saturation of the arterial blood fell considerably below normal and the cats suffered from anoxæmia, which in some experiments attained a severe degree. In contradiction to Uyeno's experiments, we find in dogs that the total ventilation per minute increased to a considerably larger extent. We never came across a case of panting in which the ventilation was increased only threefold. Even in deeply anæsthetized dogs, if the animal panted at all, the ventilation increased not less than five to sixfold, the maximum increase observed being twenty-sevenfold.

In order to determine whether there is any difference between the dog and the cat in this respect, a number of experiments were performed on cats. No fundamental difference was found in the behaviour of the dog and the cat during panting. The following experiment may serve as an average example. A cat weighing 2.25 kg. was deeply anæsthetized with urethane and exposed to heat. The rate and ventilation were continuously recorded and the stroke was calculated. With a rectal temperature of 37° C. the rate was 24, the total ventilation was 480 c.c. per minute and the stroke was equal to 20 c.c. On raising the temperature of the animal to 39° C. the rate increased to 370, the ventilation to 3450 c.c. per minute and the stroke was equal to 9.3 c.c. The ventilation was thus increased over seven times. The increase in rate and ventilation was considerably greater than that observed by Uyeno, and was comparable with the result obtained in mild panting in dogs.

The considerable diminution of stroke recorded by Uyeno is due to the fact that in his experiments he used Müller's water valves, which do not give accurate registration of ventilation at such fast rates of respiration. Moreover, the whole aim of panting is to evaporate water from the respiratory passages. Making the animal breathe through water considerably reduces the chance of efficient evaporation.

THE CARBON DIOXIDE CONTENT OF THE ARTERIAL BLOOD.

Flinn and Scott [1923] found that, on exposing non-anæsthetized dogs to high temperatures, the carbon dioxide content of the blood drawn from the jugular vein diminishes, rapidly at first and then slowly. They believe that the fall in the carbon dioxide content of the venous blood is due to the severe hyperpnœa. Their records also show that the recovery of the carbon dioxide of the blood after the exposure is slow, being still incomplete even after an interval of 2 hours. Nasset and co-workers [1931] found a diminution in the carbon dioxide content of blood and plasma in the heat polypnœa caused by diathermy. The analysis of the arterial blood of panting dogs confirms these observations. Even in mild panting, in spite of the diminution of the stroke, the carbon dioxide content of the arterial blood may fall as much as 25–40 vol. p.c. in about 1 hour. In severe panting the secondary increase in stroke leads to a further diminution of the carbon dioxide content. In some experiments the carbon dioxide was reduced by 55–60 p.c. Table III shows

TABLE III.

Time in min.	Temp. °C.	Rate per min.	Ventilation in c.c. per min.	Stroke in c.c.	CO ₂ content vol. p.c.	Lactic acid mg. p.c.
Exp. A						
0	38.0	20	750	37.5	46	27.7
50	39.9	32	1,060	33.1	43	31.5
90	40.5	240	5,000	20.8	28	39.7
110	40.5	270	6,750	25.0	28	42.2
160	37.8	34	1,000	29.4	36	27.7
200	37.5	24	912	38.0	38	27.0
Exp. B						
0	38.5	9	750	83.3	—	—
10	39.0	9	820	91.1	46	—
25	40.0	28	2,000	71.7	—	—
35	40.0	50	3,600	72.0	44	—
60	41.0	220	8,200	37.3	29	—
90	41.2	250	18,000	72.0	22	—

the results of two experiments in which the carbon dioxide content of the arterial blood happened to be the same before the onset of panting. The second animal was, if anything, more deeply anæsthetized than the

first. The difference between the two experiments was that in the first dog panting was severe, as shown by the considerable increase in the stroke. In the first case the carbon dioxide dropped in 90 min. by 39 p.c.; in the second, during the same interval of time, it dropped by nearly 55 p.c.

In the case of recovery from severe panting, the carbon dioxide content of the blood rapidly rises, with the diminution of the stroke observed during the transition from the severe to the mild form of panting, after which the recovery is slow. These changes in the carbon content of the blood are shown in Table IV. In this experiment, between the 107th and 117th min., the carbon dioxide rose from 23 to 33 p.c., as a result of the big diminution in stroke and total ventilation.

TABLE IV.

Time in min.	Temp. °C.	Rate per min.	Ventilation in c.c. per min.	Stroke in c.c.	CO ₂ vol. p.c.
0	38.0	10	530	53.0	51
20	38.9	9	600	66.7	51
40	39.5	11	680	61.8	51
55	40.5	60	1,960	32.6	49
60	40.5	140	3,750	26.1	—
70	40.5	150	5,300	35.3	40
80	41.0	240	10,620	44.3	26
107	40.0	270	12,700	47.0	23
117	39.5	190	3,600	19.0	33
140	39.0	100	1,850	18.5	41
200	37.5	13	530	40.6	44
230	36.5	12	500	45.0	45

The relation between rate, ventilation, stroke and the carbon dioxide content of the arterial blood in mild and severe panting is shown in Fig. 2.

Anrep and Cannon [1923] found that over-ventilation of an animal leads to a considerable increase in the lactic acid concentration of the blood. They consider that this is due to alkalæmia, caused by the washing out of carbon dioxide. In view of this observation it was natural to enquire as to whether in panting the slow recovery of carbon dioxide might be due to lactic acid which had accumulated during panting.

In six experiments the lactic acid was determined in the arterial blood by Clausen's method, as used by Anrep and Cannon. In all these experiments the lactic acid was found to be somewhat increased, the maximum being reached during the period of the greatest over-ventilation. In the experiment reproduced in Table III the lactic acid concentration rose from 31.5 to 42.2 mg. p.c. In the other experiments

the increase was of the same order¹. Flinn and Scott [1923] and Dadlez and Koskowski [1928] found no increase in lactic acid concentration in heat polypnoea.

In non-anæsthetized panting animals, in which the stroke appears to be very shallow, the washing out of the carbon dioxide is considerably

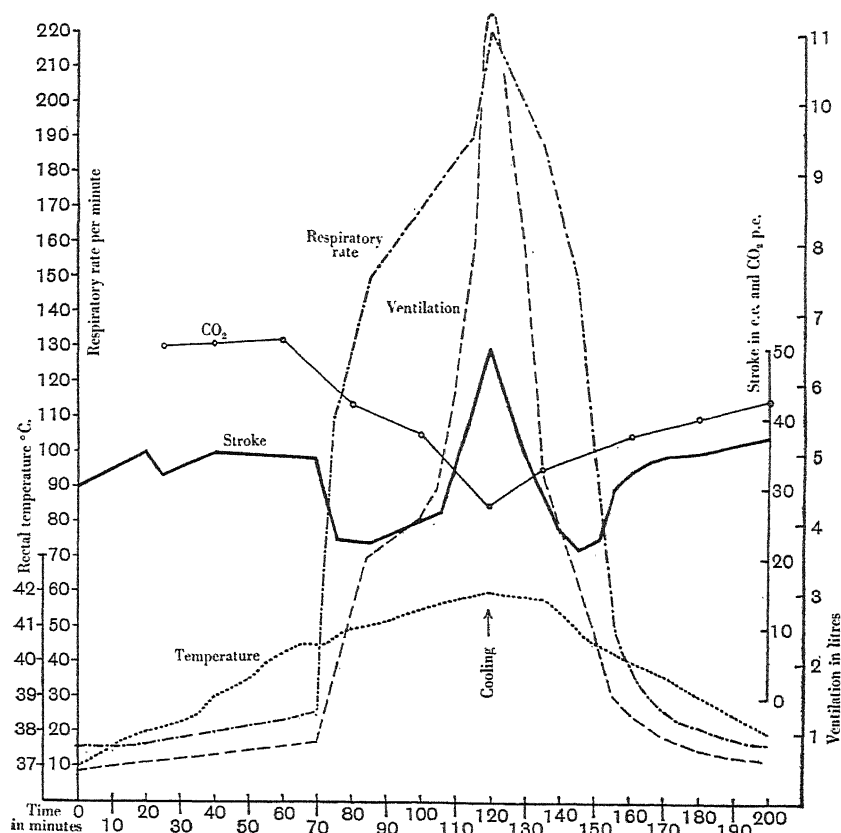


Fig. 2. The relation between the respiratory rate, ventilation, stroke and the CO_2 content of the arterial blood in panting produced by raising the body temperature.

less than in anæsthetized animals unless, as a result of long exposure to the sun, the body temperature rises appreciably and the depth of respiration increases. This is illustrated by the two following experiments. Under light anæsthesia with C. and E. mixture, a cannula was

¹ We wish to express our sincere thanks to Dr A. Hassan for having carried out the analyses of the blood samples for lactic acid.

introduced into the carotid artery, the dog was allowed to recover from the anæsthetic and left undisturbed for 2 hours. The first blood sample was then collected; the second sample was collected 50 min. later. The carbon dioxide contents were found to be 49 and 50 vol. p.c. respectively. In both cases the rectal temperature was 38.2°C . and the respiratory rate 30 per minute. The dog was then exposed to the sun. In 5 min. it began to pant at the rate of 120, and at the end of the 15th min. the rate reached 240 per minute, with a rectal temperature of 38.3°C . The arterial blood collected on the 16th min. of panting contained 44 vol. p.c. of carbon dioxide. The dog was then allowed to recover for 1 hour in the shade. The rectal temperature dropped to 37.5°C . and the carbon dioxide of the blood recovered to 50 vol. p.c. In the second experiment the carbon dioxide of the arterial blood before panting was 46 vol. p.c., the rectal temperature 38.5°C . and the respiratory rate 40 per minute. After 50 min. exposure to the sun, the carbon dioxide dropped to 30 vol. p.c., the temperature rose to 39.5°C . and the respiratory rate rose to 340 per minute. After 1 hour cooling in the shade, the carbon dioxide recovered to 44 vol. p.c., the temperature dropped to 38.5°C . and the rate dropped to 60 per minute. It was noticed that, towards the end of the exposure to the sun in the second experiment, the depth of respiration progressively increased, while in the first it remained shallow throughout.

THE EFFECT OF ADMINISTRATION OF CARBON DIOXIDE DURING PANTING.

Richet has shown that asphyxia, produced by obstructing the trachea or by considerably increasing the dead space, stops panting. This observation can easily be confirmed, but it has been found that the effect is only obtained when asphyxia reaches a considerable degree. It was of interest to find out the effect of administration of small concentrations of carbon dioxide on panting which is not complicated by anoxæmia. Table V shows the results of two experiments in which different concentrations of carbon dioxide were administered.

Small concentrations of carbon dioxide in the inspired air definitely increase the depth of respiration but have little or no effect on the rate of respiration. The total ventilation is thus increased in proportion to the increase in the stroke. Somewhat higher concentrations of carbon dioxide lead to a greater increase in depth of respiration but also diminish the rate of panting. However, in spite of the diminution of the rate, the total ventilation is increased to a much larger extent than with small

TABLE V. The period of administration of carbon dioxide is shown by heavy type. Every figure is an average of several readings. The dog's temperature was 37° C. before panting and 41° C. during panting. Panting at 40° C.

Exp. A 2 p.c. CO ₂ for 6 min.			Continuation of Exp. A 4 p.c. CO ₂ for 5 min.			Exp. B 7·6 p.c. CO ₂ for 2 min.		
Rate per min.	Ventila- tion in c.c. per min.	Stroke in c.c.	Rate per min.	Ventila- tion in c.c. per min.	Stroke in c.c.	Rate per min.	Ventila- tion in c.c. per min.	Stroke in c.c.
11	600	54·5	200	7,400	37·0	240	8,450	35·2
200	7,200	36·0	200	9,500	47·5	210	10,600	50·5
200	9,500	47·5	190	11,150	58·7	120	12,000	100·0
200	10,000	50·0	190	13,300	70·0	110	12,900	117·3
200	11,050	55·3	180	15,000	83·3	200	11,250	56·3
200	11,300	56·5	170	15,000	88·2	240	7,800	32·5
200	10,400	52·0	190	13,300	70·0			
200	9,250	46·3	200	12,600	63·0			
200	8,850	44·3	200	9,500	47·5			
200	7,400	37·0	200	710	35·3			

concentrations of carbon dioxide. With a still greater increase of carbon dioxide in the inspired air, the drop in the respiratory rate is very conspicuous so that, in spite of the fact that the stroke may be increased over threefold, the relative increase in ventilation may be less than with the smaller concentrations. Administration of carbon dioxide to vagotomized panting animals has the same effect as on animals with the vagi intact.

THE OXYGEN SATURATION OF THE ARTERIAL BLOOD.

In the literature on panting we find only a few references to the changes in the oxygen saturation of the blood. Flinn and Scott, analysing venous blood from the jugular vein, found a small increase in the percentage saturation, which they attribute to the increase of the pulmonary ventilation. This could, however, be explained by the increased rate of circulation which is observed during panting [Uyeno]. On the other hand, Uyeno found that in cats the oxygen saturation of the arterial blood falls as a result of a great reduction of the stroke. His panting animals actually suffered from severe anoxæmia, and showed in some cases a drop of oxygen saturation from 95 to 56 vol. p.c. As a rule the oxygen saturation during panting was found to be between 80 and 90 p.c. As mentioned before, Uyeno's results are most probably due to the fact that he used unsuitable valves, which did not allow of proper ventilation of the animal during the fast breathing. Experiments on panting dogs show that in no case, even in severe panting, is the oxygen saturation of the blood reduced, and this applies both to non-anæsthetized animals which are exposed to the sun and to anæsthetized

animals which are warmed in a box. Table VI shows the results of three experiments. The first was performed on a dog which was anæsthetized with a moderate dose of chloralose; the second was on a dog which was very deeply anæsthetized, and the third on a non-anæsthetized dog. The first two dogs were warmed in the box and the third was exposed to the sun.

TABLE VI.

Exp. A			Exp. B			Exp. C		
Temp. °C.	Rate per min.	Oxygen satura- tion p.c.	Temp. °C.	Rate per min.	Oxygen satura- tion p.c.	Temp. °C.	Rate per min.	Oxygen satura- tion p.c.
38.0	17	93.7	38.0	22	85.2	38.2	30	96.4
38.0	17	94.5	41.0	40	85.4	39.2	300	96.8
39.3	290	97.2	41.4	235	90.9	38.5	330	96.4
39.8	300	96.7	41.0	260	95.6	38.2	40	96.8
37.0	22	96.2	39.0	33	88.4	38.2	25	96.8
37.0	17	95.4	35.5	25	88.2			

Far from showing any anoxæmia, the lightly anæsthetized animal, whose oxygen saturation was somewhat lower than normal (probably on account of the anæsthesia), showed a small rise of the oxygen saturation. The oxygen saturation of the deeply anæsthetized animal greatly improved during panting, while in the non-anæsthetized animal the oxygen saturation remained unchanged. The oxygen capacity of the arterial blood is considerably increased in panting, a fact which is explained by previous observers as due to the considerable loss of water by the animal.

The experiments of Uyeno were performed on cats, and it seemed possible that, although dogs fail to show anoxæmia during panting, cats might behave differently. The following experiment (Table VII) shows

TABLE VII.

Time in min.	Temp. °C.	Rate per min.	Ventila- tion c.c. per min.	Stroke in c.c.	CO ₂ vol. p.c.	O ₂ satura- tion p.c.
0	Douglas' valves on					
15	39.8	260	2600	10.0	34	96.6
18	39.8	Müller's valves on				
20	40.0	260	802	3.1	—	—
35	—	240	1000	4.1	—	80.0
37	—	230	1120	4.8	43	—
38	—	240	1000	4.1	—	75.5
39	—	Müller's valves off, cat breathing without valves				
40	—	330	—	—	—	—
42	Douglas' valves on					
45	—	290	3110	10.7	—	—
60	—	320	3462	10.6	—	—
80	—	350	3350	9.6	—	98.0
90	—	330	3350	10.1	—	—
120	Cooling					
	37.5	40	650	16.3	—	—

that this is not the case. A cat weighing 2.5 kg. was anaesthetized with urethane. The total ventilation was measured in the same manner as in dogs, and the observations were made alternately with the cat breathing through Douglas' and through Müller's valves. When the anaesthetized cat was placed on the table, it was already panting, the room temperature being 34.0°C . and the rectal temperature 38.5°C . The observations began when the rectal temperature registered 39.8°C ., which happened after 45 min. of warming. Douglas' valves were then connected with the tracheal cannula. The first sample of arterial blood was collected after the animal had breathed through these valves for 15 min.

This experiment illustrates the inefficiency of Müller's valves. When used during panting they lead to a condition of rebreathing. This is further supported by the fact that the arterial carbon dioxide content increased during the period of breathing through Müller's valves, which never happened when no valves or when Douglas' valves were used.

THE EFFECT OF ANOXÆMIA ON PANTING.

Uyeno considered that the high rate of respiration obtained on exposing cats to heat is always accompanied by anoxæmia. Although our experiments show that this is not the case, it was of interest to investigate the extent to which panting is affected by anoxæmia and to compare the effect of anoxæmia with that of administration of carbon dioxide. Anoxæmia was obtained by allowing the animal to rebreathe in a closed system through a carbon dioxide absorber. The total ventilation and the rate of diminution of oxygen content of the closed system was measured graphically. The actual technique employed is shown in Fig. 3. Anoxæmia was continued until some definite change in the panting was observed, after which the dog was allowed to recover. The inspired air was not analysed, but arterial blood samples were taken at short intervals of time, and their oxygen saturation was determined. It was clear at once that the effect of anoxæmia on panting differs greatly from that of administration of carbon dioxide. Panting is not affected by anoxæmia until the oxygen saturation falls below about 80 p.c., the total ventilation and the respiratory rate remaining unchanged. As anoxæmia progresses further, the stroke gradually diminishes while the rate remains unchanged, the total ventilation obviously diminishing in proportion to the diminution in stroke. This change in depth of respiration becomes more and more conspicuous. With a further drop of oxygen saturation,

the stroke becomes progressively more and more irregular, shallow strokes alternating with periods of deeper strokes. The rate of respiration also begins to show variations, faster rates usually coinciding with deeper strokes. The rate, however, is never affected to the same extent as the

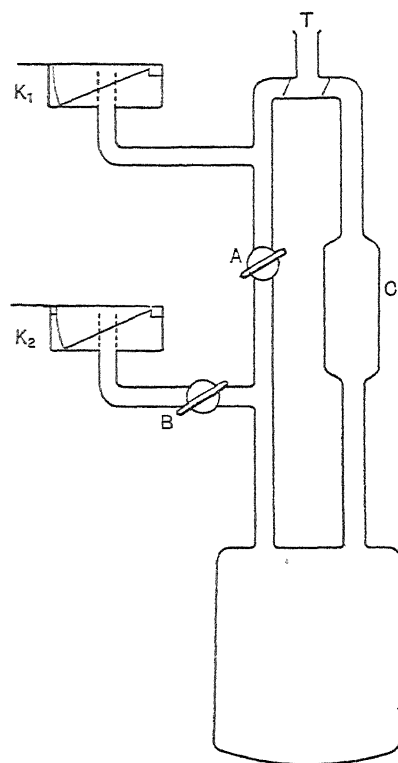


Fig. 3. Method used for determining the total ventilation of an animal breathing in a closed system. K_1 and K_2 are spirometers of 3 litres capacity each. The total capacity of the apparatus is about 15 litres. The ventilation is recorded by K_1 after closing the tap A . The animal inspires from K_2 and expires into K_1 . The CO_2 is absorbed in C . On emptying spirometer K_2 and closing tap B , spirometer K_1 registers the O_2 consumption. T is a tracheal cannula with two Douglas' valves.

stroke. These irregularities gradually become more pronounced, until the oxygen saturation of the arterial blood drops below about 45–50 p.c., when the respiratory mechanism shows a complete breakdown. Panting rapidly disappears and is replaced by slow and deep respiration, frequently of the Cheyne Stokes character. When the animal recovers from anoxæmia, the changes in rate and stroke retrace their course, but the

periodic variations remain for a long time. A comparison of the effect of anoxæmia with that of administration of carbon dioxide is shown in Table VIII.

TABLE VIII. The total capacity of the apparatus is about 15 litres.

Time	Rate per min.	Ventilation c.c. per min.	Stroke in c.c. per min.	CO ₂ content vol. p.c.	O ₂ saturation p.c.	Amount of O ₂ consumed
0	300	12,400	41.3	27	95.0	0
—	Rebreathing started					
10	300	12,000	40.0	—	—	—
15	290	10,540	36.3	31	74.9	1000
20	290	9,400	32.4	—	—	—
25	310	6,400	20.7	34	50.6	1700
30	The respiration became very irregular, panting disappeared The animal was allowed to recover for 40 min.					
0	270	12,000	44.5	27	95.4	—
1	4.5 p.c. carbon dioxide administered to the animal					
3	260	12,000	49.6	—	—	—
8	210	15,200	72.4	—	—	—
10	210	16,000	76.0	—	—	—
12	195	15,500	79.5	—	87.4	—
15	140	14,000	100.0	44	—	—
16	Carbon dioxide turned off					
25	270	12,400	46.0	23	96.4	—

THE OXYGEN CONSUMPTION DURING PANTING.

On raising the environmental temperature, the total metabolism of the animal increases as the body temperature rises. Du Bois [1921] estimated that the Q_{10} factor in prolonged experiments on man is equal to 2.3. In short experiments, according to Landis and co-workers [1926] and Koehler [1923], the Q_{10} amounts to 6.9 and 12.1 respectively, and according to McConnell and Yagloglou [1925] to 31.0. These discrepancies are explained by Bazett as being due to the pronounced hyperpnœa which accompanies the rise of body temperature. Our experiments support this hypothesis and show that, apart from the increased metabolism of the whole body, there is a large increase of oxygen consumption during panting, which can be accounted for by the increased work of the respiratory muscles. With a rise of body temperature the oxygen consumption increases in two stages; (a) a gradual rise closely following the rise of the rectal temperature up to the point of the onset of panting. In a dog of 5.5 kg., deeply anaesthetized with chloralose, the oxygen consumption rose from 50 to 70 c.c. per minute with a rise of rectal temperature from 36.5° to 40.5° C., *i.e.* a rise of 40 p.c. for 4° C. (b) A further sharp rise with the onset of panting, which is proportional to its extent, and which subsides when the animal recovers from panting. The oxygen consumption of the above dog rose to 75 and later to 85 c.c.

per minute, with respiratory rates of 100 and 190 respectively, although the temperature rose only by 0.2°C . The change in oxygen consumption which takes place during panting is shown in Fig. 4 *A*.

The experiment reproduced in Fig. 4 *A* was performed on the second day after double cervical vagotomy, which accounts for the very slow respiratory rate before panting. The results obtained on dogs with intact vagi do not differ in any way from those obtained after vagotomy.

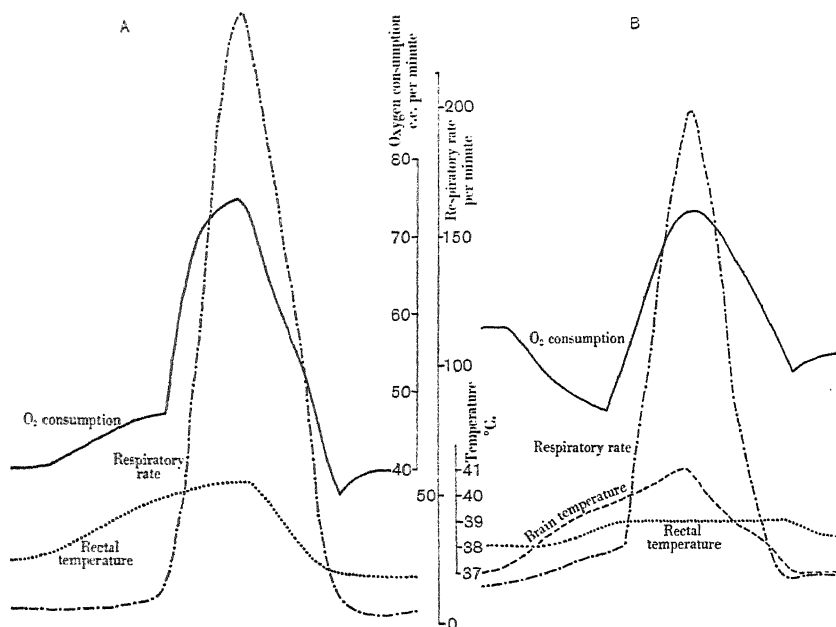


Fig. 4. The effect of panting on the O_2 consumption, (A) on warming the whole animal, (B) on warming the carotid blood. In A, the dog weighed 3.75 kg., and the experiment was made on the second day after double cervical vagotomy. In B, the dog weighed 6.5 kg., and both vertebral arteries were tied, and the carotid blood was passed through a glass spiral for the purpose of warming. The thermometer registering the temperature in the head was placed deeply under the tongue. The animal was heparinized.

In this type of experiment the body and the brain temperatures of the animal rise alike. In view of the abundant evidence in the literature that warming the brain leads to a diminution of the metabolism, the increase in oxygen consumption observed in panting, on warming the whole body, is obviously the result of several conflicting factors.

The increase of oxygen consumption in the case of panting presents,

however, no fundamental difference in animals in which the whole body is subjected to heat and animals in which panting is obtained by warming the carotid blood. The pre-panting stage, however, is different. In the first case, the oxygen consumption gradually rises with the rise of body temperature, while in the second it diminishes with the rise of brain temperature (see Fig. 4 *B*). Thus the abrupt increase of oxygen consumption, determined by the excessive activity of the respiratory muscles during panting, is in the first case accentuated by the increase in the general metabolism, while in the second case it is antagonized by the central regulating mechanism.

It has been shown by previous workers that panting does not take place after decerebration at the level of the anterior colliculi. The experiments of one of us (M. H.) confirm this observation. The respiratory rate never rises after decerebration beyond 60–70 per minute. In these animals the oxygen consumption follows the changes of the body temperature and does not show the abrupt increase characteristic of panting.

SUMMARY.

1. Panting observed on raising the body temperature was studied in dogs and cats.

2. The body temperature at which panting begins depends on the depth of anæsthesia. In lightly anæsthetized animals, panting may start at a temperature which is only slightly above normal.

3. In mild panting, the stroke is considerably smaller than normal. In severe panting, the stroke increases to normal or above.

4. In mild panting, the total ventilation is increased from six to sevenfold. In severe panting, it may be increased as much as twenty-five to twenty-sevenfold.

5. In panting, the animal is over-ventilated. The CO_2 content of the arterial blood diminishes in mild panting by 25–40 p.c. and in severe panting by 55–60 p.c.

6. Our experiments fail to support Uyenô's statement that panting animals suffer from anoxæmia. The O_2 saturation of the arterial blood in panting dogs and cats is not diminished. On the contrary if, on account of deep anæsthesia, the O_2 saturation is below normal before the period of panting, it rises during panting.

7. Administration of CO_2 to a panting animal greatly increases the stroke and somewhat diminishes the rate of respiration. The total ventilation is increased.

8. Artificially induced anoxæmia has little effect on panting until the O_2 saturation of the arterial blood falls below 80 p.c. Between 80 and 50 p.c. of O_2 saturation, the rate is almost unchanged, while the stroke shows progressive diminution. At about 50 p.c. saturation, the "respiratory panting mechanism" is put out of action.

9. The O_2 consumption during panting is increased in proportion to the total ventilation.

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THE PRESSURE OF AQUEOUS VAPOUR IN THE ALVEOLAR AIR.

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THE pressure of aqueous vapour of the alveolar air is generally accepted to be a physiological constant. In calculations of alveolar gas pressures the value 47 mm. Hg is used, a value which represents the pressure of aqueous vapour in air saturated with moisture at 37.2° C. To our knowledge no direct measurement of the pressure of water vapour in the alveolar air has ever been made, and even from theoretical considerations it would seem probable that the figure which has been accepted is too high. It would also seem possible that this value might readily show physiological variations. This is evident when we consider the assumptions made in assuming a vapour pressure of 47 mm.—It is assumed that (a) the osmotic pressure of the pulmonary epithelium is equal to that of the blood; (b) the alveolar air is always saturated with moisture; (c) the temperature of the alveolar air is 37.2° C. and does not vary. The validity of these assumptions will be discussed later.

METHODS.

Various volumetric, gravimetric and dew-point methods were tried, but we have only found the two which are described to be satisfactory.

(a) *Dew-point method.*

To obtain an accurate measurement of the temperature at which condensation of the water vapour in the alveolar air takes place, we have found the simple arrangement shown in Fig. 1 entirely satisfactory. A 500 candle-power parallel beam of light (*A*) is made to traverse a 1 litre pyrex flask (*B*) filled with water, the temperature of which can be accurately read to $\pm 0.02^{\circ}$ C. After traversing the flask the beam is reflected by a mirror (*C*) in such a way that it just misses a second mirror

(*D*) which is placed to one side of its path. The faintest trace of moisture condensing on the pyrex flask at the point of exit of the beam of light (*E*) will cause some scattering of the light rays, and some of these will impinge on the second mirror (*D*). This will show as a bright flash if the condensation be only transitory or as a steady glow if the condensation remains. The surface of the flask must be scrupulously clean (we have found that the use of concentrated nitric acid and distilled water is sufficient) and the water in the flask distilled. At the beginning of the experiment the water is heated to approximately 40° C. and then

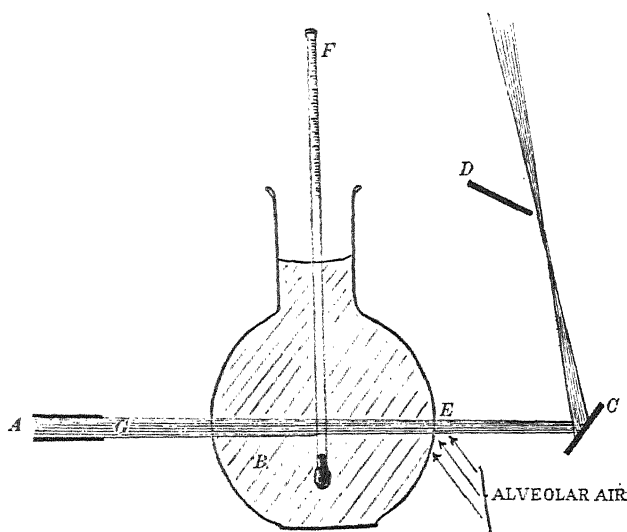


Fig. 1. Dew-point method. *A*, 500 candle-power light; *B*, pyrex flask filled with distilled water; *C*, mirror; *D*, mirror; *E*, clean surface of flask on which alveolar air is made to impinge; *F*, thermometer; *G*, parallel beam of light.

allowed to cool slowly, temperature equilibrium being ensured by continuous stirring with the thermometer. At frequent intervals samples of alveolar air are made to impinge on the pyrex flask at the point of exit of the beam of light. Whenever a flash of light is observed on the second mirror (*D*) a temperature reading is made. This temperature represents the dew point of alveolar air and from this the vapour pressure can be calculated directly. The alveolar air is made to impinge on the pyrex flask simply by making a forced expiration with the mouth close to the point of exit of the beam of light. The expiratory act should take about 3 sec., and with a little practice it is easy to stir the water, observe the mirror (*D*), and expire forcibly, at the same time.

With this system, successive measurements of the vapour pressure of the alveolar air will not vary by more than ± 0.2 mm. of Hg. To form some estimate of the absolute accuracy of the method we have measured the vapour pressure of air artificially saturated with moisture. Air is passed through a water pumice stone tower, its temperature is accurately measured, and it is then allowed to impinge on the pyrex flask. The temperature of the air was always found to be somewhat higher than the temperature of the flask at which condensation was observed (Fig. 2), the discrepancy amounting to an average of 0.23°C . in 57 observations. Unfortunately, since the evaporation of water produces an appreciable lowering of the temperature in the saturating chamber, it is impossible with this system to have complete temperature

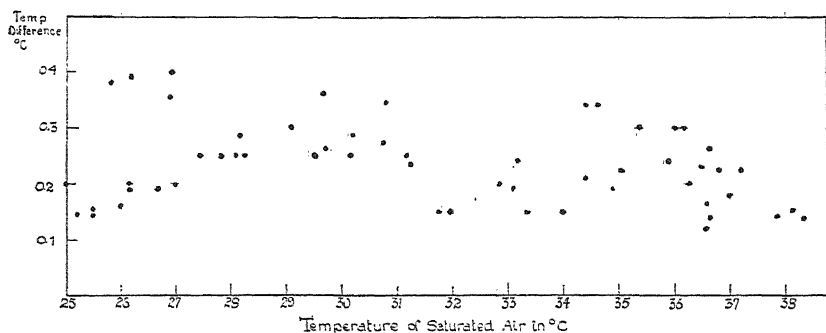


Fig. 2. Calibration of dew-point method. Showing difference between temperature of dew formation and temperature at which air was saturated with moisture.

equilibrium between the air saturated with moisture and its environment. For this reason we strongly suspect that some at least of the variations shown in Fig. 2 are due to unsaturation of the air caused by increase in temperature after saturation. This supposition is strengthened by the fact that a much smaller variation was obtained when air saturated with water vapour in the lungs was used. However, the factor 0.23°C . certainly represents the maximum difference between the true dew point and the observed dew point by this method, and as such has been used as a constant correction factor.

(b) Gravimetric method.

To measure the weight of water in a known volume of alveolar air we have passed successive Haldane-Priestley samples of alveolar air through a chain of P_2O_5 tubes and then carefully measured the total volume of alveolar air by water displacement.

A three-way glass tap of 4 mm. bore is wound with several yards of insulated German silver wire and the whole bound with asbestos string. By passing a 12-volt current through the wire the tap can be heated to well above body temperature. One limb of the tap is covered with rubber so as to serve as a mouthpiece and so placed that by turning the tap expiration may take place through one or other of the airways. One of these is left open to the atmosphere and the other directly connected to two large P_2O_5 tubes in series, filled with P_2O_5 and glass-wool. After having traversed these tubes the air is led through a gas sampling tube of 1 cm. bore and 25 cm. length, followed by a tower filled with pumice stone saturated with 1 p.c. sulphuric acid to saturate with water and avoid absorption of CO_2 , and finally a graduated cylinder capable of measuring the gas volume to within ± 2 c.c. by water displacement. This cylinder is equipped with a thermometer to measure the temperature of the gas, and a water manometer to ensure pressure equilibrium between the gas whose volume is being measured and the atmosphere. Instead of water, 1 p.c. H_2SO_4 is used for displacement to avoid absorption of CO_2 . The technique of analysis is as follows. The P_2O_5 tubes are carefully dried and weighed to ± 0.1 of a mg. The gas chain is then assembled as described above and the mouthpiece tap heated by passing sufficient current through the German silver wire. The mouthpiece tap is turned so that the airway is from mouthpiece to room air. The H_2SO_4 reservoir is lowered so that a negative pressure of 20 cm. of H_2O is developed in the system. Flow of water between the reservoir and the graduate cylinder is then prevented and, by observing the level in the water manometer, the presence of any leak detected. If no leak occurs the experiment is begun and a negative pressure of 20 cm. of H_2O throughout the system maintained. A deep breath is taken and the lips firmly applied to the mouthpiece on the three-way tap, which is heated to a temperature which can just be tolerated. The breath is held for 20 sec. and a slow expiration lasting from 3 to 5 sec. made, so as almost but not completely to expel the complementary and reserve air. The three-way tap is then turned so that from 100 to 150 c.c. of the air from the lungs are drawn through the gas chain. This procedure is repeated until 5 litres of alveolar air have been measured by the measuring cylinder. The P_2O_5 tubes are again weighed and the vapour pressure calculated from the usual formula $PV = \frac{W}{M} RT$. The following points are important. As the sample of alveolar air which is drawn through the gas chain remains in the mouth for several seconds, breathing must be through the nose during and prior to each deep breath. No saliva must

be allowed to touch the limb of the three-way tap in the mouthpiece. There must be no condensation of moisture in the arm of the P_2O_5 tube within 2 cm. of its termination.

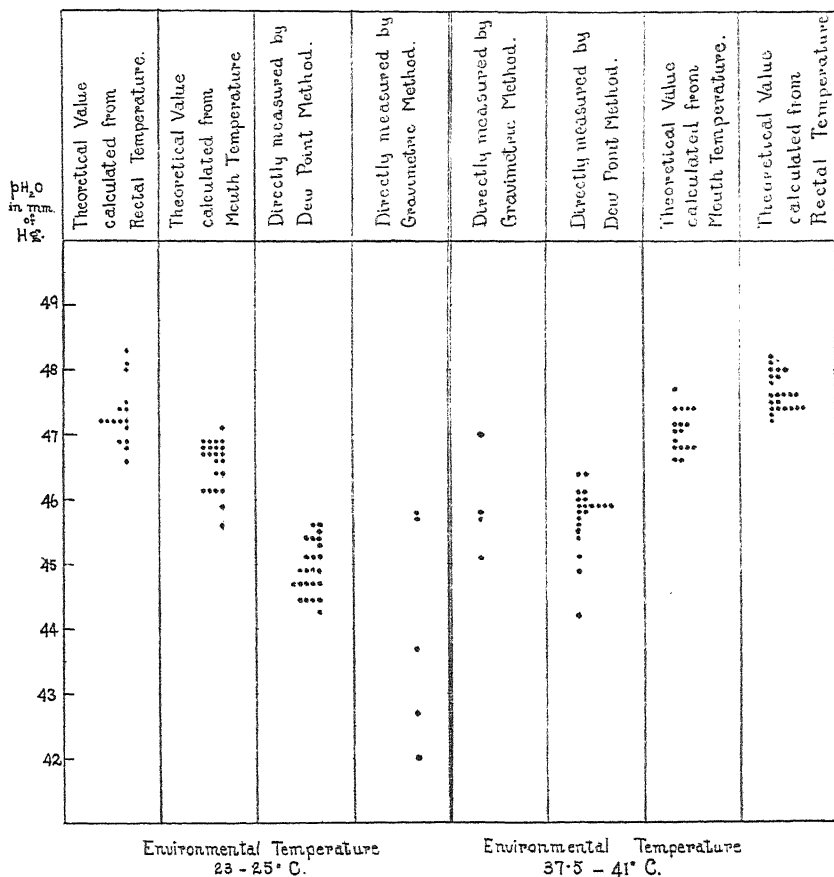


Fig. 3. The vapour pressure of the alveolar air by direct measurement and by calculation from the rectal and mouth temperatures.

In spite of the utmost care the figures obtained for the vapour pressure of the alveolar had a range of almost 3 mm. of Hg, which compares unfavourably with those obtained by the dew-point method (Fig. 3). Also an observation by this method takes 2 hours or more, while by the dew-point method only from 2 to 5 min. are required.

RESULTS.

As might be expected the vapour pressure of the alveolar air was found to follow closely changes in the systemic temperature. For the sake of clarity we have calculated the vapour pressure of air saturated with moisture at the rectal and mouth temperatures, and compared these pressures with those obtained by direct measurement on the alveolar

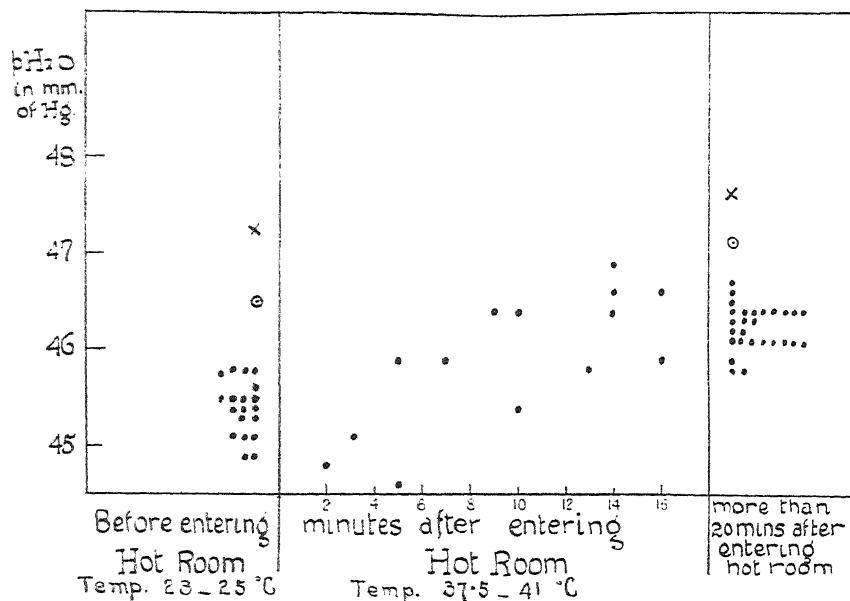


Fig. 4. The vapour pressure of alveolar air brought into equilibrium with the venous blood by 45 sec. apnoea. Measured before and after entering hot room, by dew-point method.

x = vapour pressure calculated from average rectal temperature.

o = vapour pressure calculated from average mouth temperature.

• = vapour pressure measured by dew-point method.

air. The results are shown in detail in Fig. 3. The theoretical values calculated from the rectal and mouth temperatures average 47.26 and 46.55 mm. respectively, whereas the measured values average 44.91 mm. by the dew-point method and 44.0 by the gravimetric. When the temperature of the environment was raised to between 37.5 and 41° C. the vapour pressure calculated from the rectal and mouth temperatures rose to average values of 47.65 and 47.16 mm. respectively, followed by the alveolar air which rose to 45.81 mm. by the dew-point method and 45.9 by the gravimetric (Fig. 3).

Effect of voluntary apnoea.

When complete equilibration between blood and alveolar air was ensured by holding the breath for a period of 45 sec., the same change was found to take place, the vapour pressure rising from an average of 45.43 mm. in the cold environment to 46.26 in the hot (Fig. 4). In both hot and cold environment the apnoea produced *per se* an average rise of 0.5 mm. in the alveolar vapour pressure (Figs. 3 and 4).

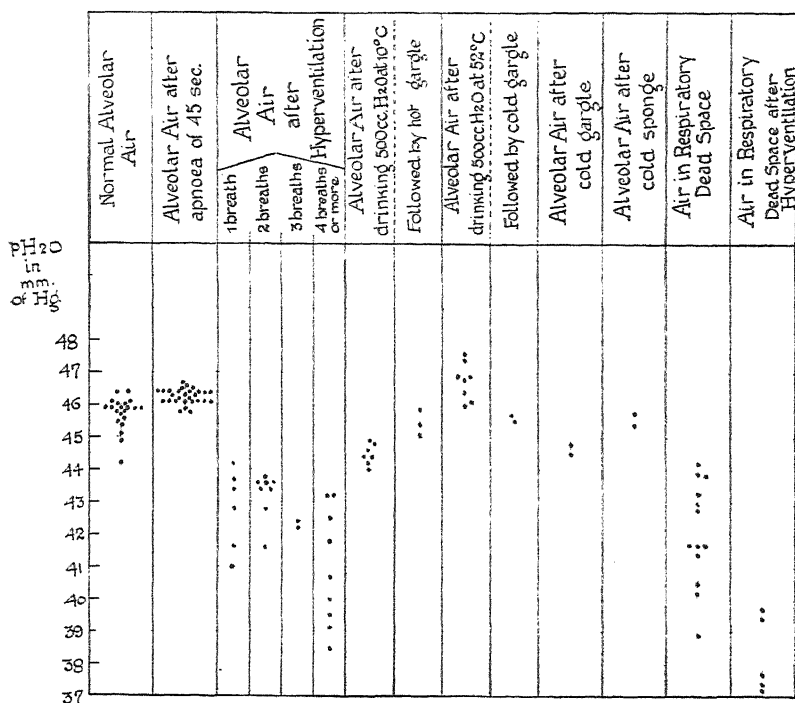


Fig. 5. Variations in the vapour pressure of the alveolar and dead space air. Dew-point method. Temperature of environment 37.5–41.0° C.

Effect of hyperventilation.

One full inspiration immediately followed by a forced expiration is sufficient to produce a perceptible lowering of the vapour pressure of the alveolar air as measured by the dew-point method (Fig. 5). The average values found were 42.8 mm. after one deep breath, 43.2 mm. after two breaths, 42.3 mm. after three breaths and 40.9 mm. after four

or more breaths. The lowest value obtained was 38.5 mm. after eight deep breaths. These experiments were all conducted in an environmental temperature of from 39 to 41° C.

Rapid and shallow breathing.

After voluntary tachypnoea of 45 sec. duration with a respiratory rate of 60 a minute and depth of approximately 200 c.c., the vapour pressure of the expired air at the end of such a shallow breath amounted to an average of 38.3 mm. (Fig. 5). These experiments were also conducted in an environmental temperature of from 39 to 41° C.

Effect of cooling mouth and naso-pharynx.

If the vapour pressure of the alveolar air is measured before and after 500 c.c. of water at 12° C. are drunk, a slight but definite fall is observed, amounting to an average of 1.3 mm. (Fig. 5). In the same way the imbibition of hot water causes an average rise of 1.0 mm. Similar changes were observed on air held in the lungs for 45 sec. Either of these effects can be eliminated by a hot or cold gargle following the cold or hot drink respectively (Fig. 5). The duration of this apparent lowering of the alveolar vapour pressure was followed on four occasions after cold water was drunk and it was found that 8, 8, 6 and 4 min. respectively had to elapse before a normal reading could be obtained on either the alveolar air, or air held in the alveoli for 45 sec.

Vapour pressure of air in the respiratory dead space.

The vapour pressure of the expired air after only 100 c.c. had been expired was found to average 42.1 mm. (Fig. 5). After an apnoea of 30 sec. the vapour pressure of this air was found to have mounted to 44.4, 44.9, 45.1 and 45.1 mm. on four estimations.

CRITICISMS.

These experiments, especially those with hot and cold water, immediately suggest that under any conditions, whether with quiet respiration or with hyperventilation, the alveolar air may be cooled during its passage from the alveoli to the mouth. Were this true any application of the dew point as measured by this method to the vapour pressure of the alveolar air would be entirely fallacious. One purpose of the experiments which have been described was to prove or disprove this possibility, and, when we analyse the evidence, it would seem to be entirely against such a supposition.

(a) Rapid and shallow breathing produces no lowering of the vapour pressure of the true alveolar air. Neither rapid and shallow breathing nor hyperventilation produced any lowering of the vapour pressure of air subsequently held in the lungs for 45 sec. With cold water, on the other hand, the lowering of the dead space temperature sufficient to produce a fall in vapour pressure of only 1 mm. persisted over a period of from 4 to 8 min.;—obviously a different mechanism from that which produced a very much greater but purely transitory fall in vapour pressure.

(b) Were the alveolar air being cooled by the respiratory passages one would expect this effect to be greater when breathing cold air than hot. It follows that a greater rise in the vapour pressure of the alveolar air is to be expected from a period of apnoea after breathing cold air than after breathing hot air. Within the limits of experimental error this was not found to be the case, the rise with cold air amounting to an average of 0.5 mm. and with hot air to an average of 0.45 mm., in twenty observations.

(c) With the exception of the group of observations on the normal vapour pressure of the alveolar air, all the experiments described were performed in an environmental temperature above that of the body. The bronchi were covered with mucous of high viscosity, and it would indeed be surprising if under these circumstances they could be perceptibly cooled by any form of respiratory gymnastics.

DISCUSSION.

This is the first attempt, as far as we know, which has been made to measure directly the vapour pressure of the alveolar air. Mainly in the course of studies on water balance several attempts have been made to measure the total amount of water excreted by the lungs over a given length of time. In all some gravimetric method has been used in none of which is the possibility of condensation of moisture outside the weighing system completely excluded. From this point of view each method has its own particular possibility of error and we will only discuss in brief the conclusions which have been drawn. All are agreed that the expired air is far from saturated with moisture [Galeotti, 1912 and 1915; Loewy and Gerhartz, 1914; Jacquot and Mayer, 1925, 1926], but that the saturation rises with the temperature of the environment [also Rubner, 1898], and is unaffected by the humidity of the environment [also Levi, 1925, *a*, *b*]. Viale [1926] has shown that deep breathing increases water excretion of the lungs, and Galeotti

and Signorelli [1912] have shown the water excretion is increased at high altitudes. From our own results these conclusions would seem to be perfectly reasonable, but it must be remembered that water excretion is largely a function of the volume of air expired, and does not necessarily bear any relationship to the vapour pressure in the alveoli.

Evidently the vapour pressure of the alveolar air is lower by some 2 mm. than the generally accepted value of 47 mm. of Hg. From the experiments described we are able to analyse in some detail the factors responsible for this discrepancy. (a) Holding the breath for 45 sec. raises the vapour pressure of the alveolar air by 0.5 mm. irrespective of the temperature of the environment. The possibility of any significant cooling of the alveolar air while passing through the respiratory dead space has been excluded, so we can assume that 0.5 mm. represents the lack of equilibrium between the blood and the alveolar air. Such a gradient seems very reasonable when we consider that Bock and his associates [1924, 1929-30] have shown that with CO_2 a measurable gradient exists between the plasma and the alveolar CO_2 . (b) The difference between the osmotic pressure of the blood and that of water could only account for a lowering of the vapour pressure of at the most 0.15 mm. (c) Binger and Christie [1927] in a large number of observations have shown that, in the anaesthetized dog at least, the lung temperature is always lower than that of the rectum. We have taken 50 such observations at random and have found that the lung temperature is on an average 0.24°C . lower than the rectal temperature. The temperature was measured by thermocouples accurate to $\pm 0.02^\circ \text{C}$. In the same way we found that the blood passing through the lungs was cooled by an average of 0.08°C . on seven simultaneous measurements of the temperature of the right and left heart blood [Binger and Christie]. We have ample evidence then for the assumption that the temperature of the lung is somewhat lower than that of the rectum. If we accept a difference of 0.24°C . as also applying to the human subject, this would represent a fall of 0.6 mm. in the vapour pressure.

These three factors could account for 70 p.c. of the discrepancy between the vapour pressure of the alveolar air, as measured by us, and that which has been assumed in the past. When we consider that there is a difference of about two atmospheres between the osmotic pressure of the yolk and white of an egg [Straub, 1930; Hill, 1931], it would indeed be remarkable if all the factors influencing even the vapour pressure at the surface of a living cell could be analysed.

Effect of environmental temperature.

Even with extreme changes in the temperature of the environment the vapour pressure of the alveolar air was found to follow closely the temperature of the rectum. The same was found to be true with air retained in the lungs for 45 sec. In both the vapour pressure reaches an equilibrium after some 5 to 10 min., while 15 min. or more is necessary for constant readings of the rectal temperature (Figs. 4 and 5). This early rise in the vapour pressure, unaccompanied by any change in the rectal temperature, at first deceived us into the rather attractive hypothesis that a change in the osmotic pressure of the blood and pulmonary epithelium was being reflected. However, the rise in vapour pressure is far greater than could be produced by any such change, and when we take into consideration the lag of the rectal temperature, the phenomenon can be explained more simply by a rise in the temperature of the lung.

Effect of cold and hot water.

It has been suggested [Galeotti, 1912, 1915; Jacquot and Mayer, 1926] that there is some reflex mechanism in the lungs to increase or decrease water excretion, the stimulus being a sensation of cold or warmth. We have been unable to find any evidence for such a reflex. The changes in vapour pressure which we observed with changes in the environmental temperature have already been discussed, and the changes after imbibition of cold or hot water have been shown to be due probably to changes in the temperature of the respiratory dead space.

Effect of hyperventilation.

The lowering of the alveolar vapour pressure which was found after hyperventilation might either be due to cooling of the lungs or to an increased gradient between the alveolar air and the blood. The possibility of cooling the respiratory air passages has already been disposed of, and it seems inconceivable that cooling of the lung could account for the change observed. During the 10–15 sec. period of hyperventilation certainly not more than 0.4 g. of water is excreted, which represents a maximum heat loss of 230 calories, a quantity quite insufficient to lower the temperature of the lung and blood circulating through it by the 3° C. necessary for such a fall in vapour pressure. The lung probably is cooled to a slight extent, but the factor mainly responsible for the fall in vapour pressure must be lack of equilibrium between the alveolar air and the blood, the gradient probably being one of temperature as

well as saturation. Whatever factors may be responsible, the fact remains that the alveolar vapour pressure may be lowered by as much as 9 mm. of Hg by hyperventilation, even in an atmosphere which is warmer than that of the lungs.

This observation would seem to have a direct bearing on any accurate analysis of hæmo-respiratory exchange during work or at high altitudes. With a fall of the alveolar vapour pressure to 39 mm. an error of 0.5 mm. in the pressure of CO_2 and of 1.1 mm. in the pressure of oxygen would result from the use of the standard value of 47 mm. At an altitude of 15,000 ft., with an alveolar oxygen tension of 50 mm. and CO_2 tension of 30 mm. [*Report of the Peru High-Altitude Committee, 1923*], this would result in an error of 0.5 p.c. in the calculated saturation of the arterial blood and an error of 0.01 in the $p\text{H}$ calculated from the Henderson-Hasselbach equation.

Rapid and shallow breathing.

With a respiratory rate of 60 a minute and depth of 200 c.c. the pressure of water vapour at the end of such a breath was found to average 38.3 mm. Hg, the pressure in the alveoli remaining unchanged. Under similar conditions the tension of CO_2 was found to be 23.2, 22.4 and 20.1 mm. on three occasions, with a CO_2 tension of the expired air of 8.9, 10.0 and 7.8 mm. respectively. The alveolar $p\text{CO}_2$ of this subject averaged 41 mm. on eight observations.

It follows that rapid and shallow breathing is a very efficient mechanism for the elimination of heat by the evaporation of water with CO_2 loss reduced to a minimum. Were the loss of CO_2 to parallel the loss of H_2O a gaseous alkalosis would soon result. In cases of lobar pneumonia with rapid and shallow breathing this does not occur and a dog does not develop tetany when he pants. Presumably this lack of parallelism is to be explained on the score of evaporation in that region where the dead space ends and the alveolar air commences. The evidence we have presented is certainly against any significant lowering of temperature in the respiratory tract and, *a priori*, it would seem that heat loss must be from the pulmonary vascular bed, the whole structure of which, with its amazingly efficient "water-cooled" system, would lend itself to the evaporation of moisture without any significant fall in temperature. When we consider the lack of agreement which exists between what might be called the Haldane school on the one hand, and the Krogh school on the other, as to the boundaries and even significance of the respiratory dead space, it is easy to conceive an area between the

anatomical dead space and the alveoli, hyperventilation of which would produce a loss of water out of proportion to the loss of CO_2 . Indeed, if we accept the evidence of Krogh and his associates against homogeneity throughout the alveolar air, such a possibility seems likely, since more H_2O vapour than CO_2 can be given off by a cubic centimetre of blood when hyperventilated. Moreover, in this area heat loss would be from the pulmonary blood itself and not from the bronchial capillaries. Such a mechanism would fully explain the absence of gaseous alkalosis in such conditions as lobar pneumonia, the panting of dogs and any form of thermal polypnoea. It would also explain the subnormal temperature usually found in cases of emphysema with chronic rapid and shallow breathing, with impairment of the function of CO_2 excretion. In repeated observations on five such cases we have found an average temperature of 36.4°C .

SUMMARY AND CONCLUSIONS.

1. A method for the accurate measurement of the vapour pressure of the alveolar air is described.

2. The vapour pressure of the alveolar air is some 2 mm. Hg lower than the value generally accepted. An explanation for this discrepancy is given.

3. The vapour pressure of the alveolar air can be lowered by hyperventilation by as much as 7 mm. Hg. The significance of these observations with regard to hæmorepiratory exchange during conditions of work and at high altitudes is discussed.

4. Changes in the alveolar vapour pressure with the temperature of the environment can be fully accounted for by changes in the systemic temperature.

5. The efficiency and specificity of rapid and shallow breathing as a mechanism for the elimination of heat and conservation of carbon dioxide is described. The application of this mechanism to thermal polypnoea, lobar pneumonia and emphysema is discussed.

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STUDIES IN MUSCULAR ACTIVITY.

VII. Factors limiting the capacity for work.

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In a dog the energy reserve consists chiefly of carbohydrate and fat. For their utilization oxygen must be supplied and carbon dioxide eliminated. The maximum over-all efficiency is probably from 20 to 30 p.c., most of the energy appearing as heat. Hence, if a constant body temperature is to be maintained, provision must be made for heat dissipation. The experiments to be described indicate that, by suitably varying the conditions, inadequacy in any one of the three factors, fuel supply, oxygen supply or heat dissipation, may limit the capacity for work. In our experiments these three taken singly, or in combinations, are of primary importance, although factors of secondary importance no doubt exist.

Two dogs were trained to run on a motor-driven treadmill. Most of the experiments were carried out on Joe, an immature male of the fox-terrier type weighing 13 kg. Additional experiments were carried out on another dog, a mature female of the Irish terrier type and of the same weight. With the exception of a few early experiments the grade¹ was 17.6 p.c. The rates and other experimental conditions will be given in detail below. Observations were made of: (a) heart rate, using a cardiometer previously described; (b) rectal temperature either with a thermocouple during exercise or with a clinical thermometer after it; (c) room temperature; (d) blood lactic acid by the method of Friedemann, Cotonio and Schaffer [1927]; (e) blood sugar by the method of Folin and Malmros [1929]; and (f) morphological properties² of the blood. Notes were made of the dog's behaviour, particularly during the onset of exhaustion. The nomenclature of Campos, Cannon, Lundin

¹ According to Tracy [1908] a grade of 1 p.c. is defined as "a rise or fall of 1 ft. measured *vertically* for each 100 ft. measured *horizontally*." This usage, generally accepted by engineers, has been adopted in our calculations although it is not always followed by physiologists, e.g. by Smith [1922].

² These will be described elsewhere.

and Walker [1929] will be followed in describing the degree of fatigue. The relative humidity was low in all cases, and with a few exceptions (all at low temperature) there was virtually no air movement.

The blood samples were all obtained from the saphenous vein as soon as possible after work stopped. Usually not more than a minute elapsed before the sample was withdrawn. Christensen [1931] suggests that such samples should be called recovery rather than work samples. He shows that the blood-sugar level in man may increase several mg./100 c.c. during the first few minutes of recovery. Three experiments may be cited which show that under the conditions of our experiments only a small change occurs during the first minute of recovery, and hence our values for blood sugar differ but little from work samples. In the first case, a cardiac puncture was made while the animal was running. As soon as blood was obtained, the dog was lifted from the treadmill and with the needle remaining in position a series of samples was collected. In each of two experiments on Joe, one in hypoglycæmia and another in a normal state, a series of samples was obtained during recovery. These results, assembled in Table I, show that changes in blood sugar during the first minute of recovery are unimportant from our point of view.

TABLE I. Change in blood-sugar concentration during recovery.
Zero time corresponds to cessation of work.

Experiment 1		Experiment 2		Experiment 3	
Time (sec.)	Blood sugar mg./100 c.c.	Time (sec.)	Blood sugar mg./100 c.c.	Time (sec.)	Blood sugar mg./100 c.c.
- 10	86	15	46	10	106
15	92	40	52	30	105
60	97	100	53	90	110
120	92	120	52	130	121
240	92	270	49	160	119
—	—	330	47	—	—
—	—	360	55	—	—

The experiments illustrated in Fig. 1 include several in which either the supply of oxygen or the dissipation of heat determined the work output. In one of these experiments the rate of oxygen supply certainly was the chief limiting factor. In this case, with a room temperature of 16° C., the energy output during work was 352 kg.m. per minute¹ for 7½ minutes, a total of 2640 kg.m. At the end of work there was evidence of a large

¹ The energy output has been calculated from the data of Slowstoft [1903]. He found that the energy requirement for running on an inclined treadmill may be resolved into horizontal and vertical components. For dogs of 12-14 kg. weight the horizontal component is 0.64 kg.m. per metre, and the vertical component 2.92 kg.m. per metre. These are expressed in kilogram-metres per kilogram of body weight.

oxygen debt. The blood lactic acid concentration was high, 74 mg./100 c.c. The laboured and deep character of the respiration during recovery indicated that a high rate of oxygen intake was being maintained. There was confirmatory evidence of an indirect nature. Thus, the rectal temperature

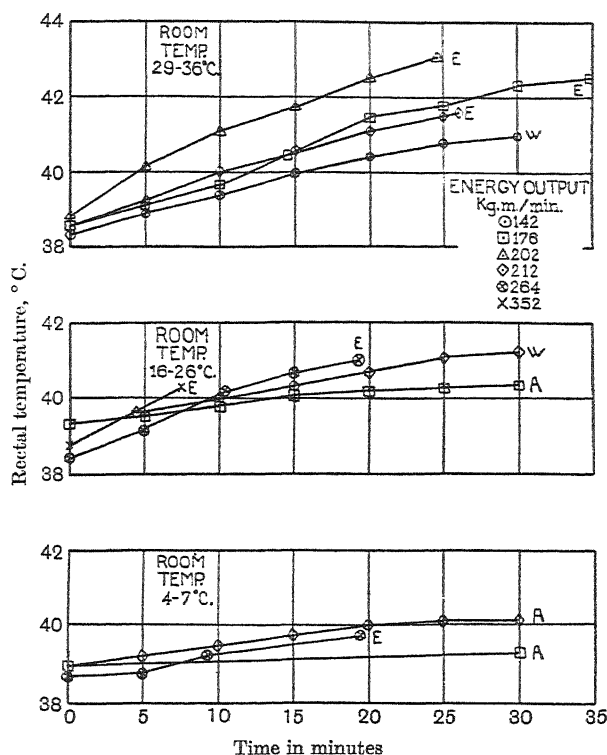


Fig. 1. Rectal temperature in relation to duration of work, intensity of work and room temperature. Exercise was continuous, temperatures being observed with a rectal thermocouple. The degree of fatigue is indicated by the notation of Campos, Cannon, Lundin and Walker [1929]. A=Active; ready to continue running. W=Weary; panting, but easily able to continue running. E=Exhausted; panting most heavily with head down, drops to platform and refuses to rise.

was raised only 1.5° C., an increase which is small in comparison with changes in other experiments shown in the same figure. Furthermore, the blood sugar remained at a high level, 119 mg./100 c.c. Failure was not because of high temperature or lack of fuel. In view of the direct evidence and since no other cause for exhaustion is known, it seems probable that this dog cannot transport enough oxygen to remain in a steady state at

this rate of work. Energy reserves were utilized anaerobically, resulting in accumulation of unoxidized end products. It may be noted in passing that lactic acid accumulation alone is inconclusive evidence of oxygen deficiency in the dog. This subject will be referred to below.

In some of the experiments illustrated in Fig. 1, conditions were adjusted so that inadequate heat dissipation was the primary limiting factor. One of the necessary conditions for this is that the demand for oxygen shall not exceed the transport capacity. While the maximum rate of energy output which this dog can maintain in a steady state has not been determined, a single experiment may be cited which proves that his working capacity is not less than 212 kg.m. per minute. He maintained this output without interruption for 112 minutes, the linear velocity being 187 metres per minute and the grade 17.6 p.c. The room temperature ranged from 4° to 7° C. and at the end his rectal temperature was 39.3° C. A sample of blood drawn within a minute after work stopped was found to contain 105 mg./100 c.c. of sugar and 12 mg./100 c.c. of lactic acid. Apparently, in respect of body temperature, blood sugar and blood lactic acid he remained within the limits which are normal for the resting state, notwithstanding the fact that his oxygen intake was perhaps 15 times the resting level.

This experiment was repeated with all conditions the same except room temperature. At 20° C. he ran for 29 minutes with the rectal temperature rising gradually to 41.3° C. Although weary at the end, he could have gone farther. At 33° C. he ran to exhaustion in 27 minutes with a final temperature of 41.6° C. With a somewhat higher room temperature, 35° C., but with easier work, 176 kg.m. per minute, he ran for 34½ minutes to exhaustion with a terminal temperature of 42.5° C.¹ The highest rectal temperature reached, 43.1° C., was at a room temperature of 29° C. with an energy output of 202 kg.m. per minute for 24 minutes. This was an early experiment with a rate of 233 metres per minute and a grade of 8 p.c. These results prove that work which is easy at low temperatures is difficult at moderate temperatures. As the external temperature is increased, the length of time a given rate of energy output can be maintained diminishes.

There are many indications that exhaustion resulting from work at high temperature is a complex phenomenon. One notes the increase in

¹ In an experiment to be described below (Protocol I), this rate of energy output was maintained for 17 hours with the room temperature near 15° C. and with fuel and water supplied during half-hourly rest periods. A difference of room temperature of 20° C. determines the difference between a steadily increasing body temperature ending in exhaustion and a constant slightly elevated temperature with virtually tireless performance.

effective area from which heat is dissipated and simultaneous increases in salivary flow, respiratory rate and respiratory volume. These changes call for a greater oxygen supply both for the muscles involved in breathing and for the heart. With a rate of oxygen supply already near the

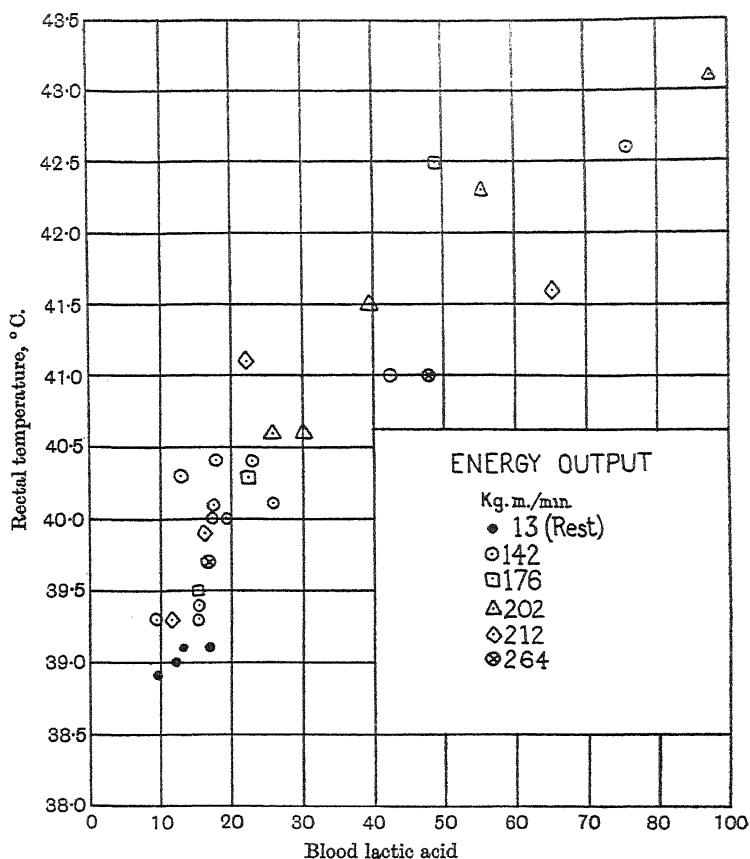


Fig. 2. Blood lactic acid in relation to rectal temperature. The blood sample was drawn at the end of a work period of at least 15 minutes' duration and rectal temperature was observed simultaneously.

upper limit, this further demand may result in breakdown. Under such conditions, before the rectal temperature has reached a high value, exhaustion may come as a consequence of anaerobic production of mechanical energy for dissipating heat. At present the evidence for this statement must remain indirect since, as intimated above, lactic acid

concentration is not an infallible measure of oxygen debt in the case of the dog.

The highest rectal temperatures have been reached with rates of oxygen intake well below the maximum. In such cases, as body temperature rises, polypnoea and related changes become more pronounced. This forced breathing removes CO_2 faster than it is formed. Under similar conditions Rice and Steinhaus [1931] have observed CO_2 pressures in blood as low as 10 mm. While the results of Bock, Dill and Edwards [1932] indicated that men do not readily respond to overventilation by forming lactic acid, it seemed likely that the dog might do so. The experiments of Knudson and Schaible [1931] suggest that such is the case with resting dogs. It is therefore of interest to enquire whether in the dog, as part of the response to work at high temperature, formation of lactic acid occurs.

The results presented in Fig. 2 show that lactic acid concentration does not increase until rectal temperature exceeds 40°C ., but that between the limits of 40°C . and 43°C . and over a wide range of metabolic rates, it increases with rectal temperature. This result is in harmony with Knudson's observations on dogs at rest. It appears that lactic acid accumulation in the dog is not necessarily due to anoxemia. If the work is hard enough lactic acid may accumulate directly from oxygen lack as in man, and if the temperature is normal its concentration is more or less proportional to the oxygen debt. In rest, however, or even in moderate work, it may accumulate as part of the response of the heat dissipative mechanism¹. In such a case a high concentration of lactic acid should not be considered as indicating incapacity for transporting more oxygen even though in recovery removal of lactic acid is accomplished as usual, viz. by aerobic resynthesis.

Up to this point the limiting factors considered have been oxygen supply and heat dissipation. What happens if the work is moderate and the temperature low? First of all the animal must have water; provided water is supplied, what determines the capacity for work? The limiting

¹ The results of Rice and Steinhaus [1931] indicate a reduction in alkali reserve during work accompanied by increase in rectal temperature. It is possible with certain assumptions to calculate the decrease in alkali reserve (the total CO_2 of oxygenated blood when $p\text{CO}_2=40$ mm.) from their data. Thus in their Fig. 1 the $p\text{CO}_2$ was reduced from 40 to 10.5 and the (BHCO_3) from 24 to 6.7 mM/litre. Assume the same oxygen content and an oxygen capacity of 20 volumes per cent. in these blood samples. On the basis of these assumptions and the known properties of dog blood [see Dill, Edwards, Florkin and Campbell, 1932] the reduction in alkali reserve was about 7 mM. Since the pH was nearly constant such a change in alkali reserve implies an increase in blood lactic acid of about 60 mg./100 c.c.

factor seems to be merely the quantity of easily available fuel; the principal evidence for this is found in Fig. 3. These experiments were carried out according to a uniform plan. The run began either after a 36 hours' fast or 1 hour after a heavy carbohydrate meal. In the first case, exercise was carried on to exhaustion; in the second case, fuel was

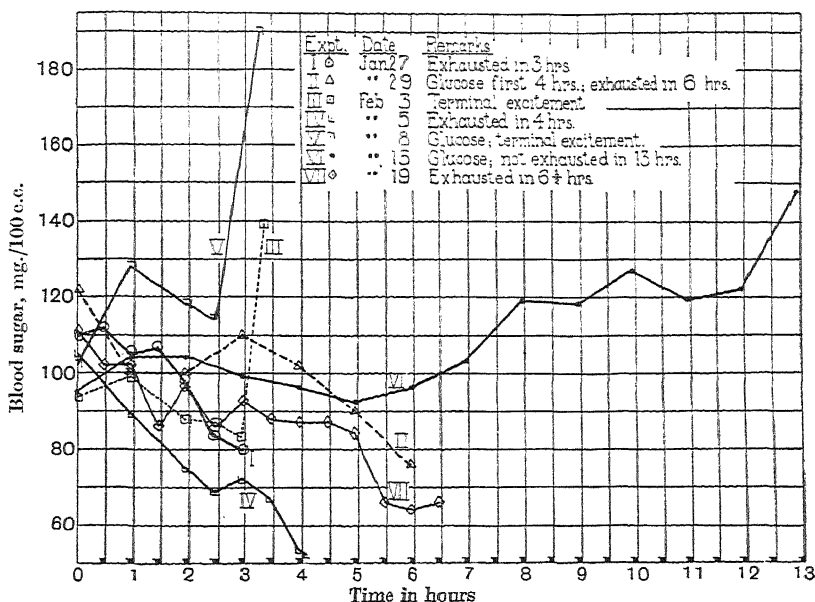


Fig. 3. Performance in relation to blood sugar. Observations were made at 30-minute intervals during rest periods of 5 minutes' duration.

supplied at intervals¹. In either case the schedule called for 25-minute runs with intervening periods of 5 minutes for withdrawing blood, observing rectal temperature, supplying water and, in some cases, fuel. The

¹ The fuel used (except in one case when sterile glucose was injected and in a second case when glucose was given by stomach tube) was a candy referred to as glucose candy. Analysis was made after the experiments were concluded and it was found to contain 16 p.c. glucose and 72 p.c. sucrose + polysaccharide. According to the manufacturer it also contained citric acid and artificial colour and flavour. It contained by analysis 0.17 p.c. ash and less than 1 mg. of combined phosphorus per 100 g. The dog is able to utilize this fuel alone during long periods of exercise, indicating that added phosphate is unnecessary, contrary to the suggestion of Dische and Goldhammer [1932]. Furthermore, in order to utilize sucrose the secretion of digestive juices is necessary. For this purpose it is particularly important to maintain a low temperature. Nausea and vomiting are likely consequences of running a dog with a full stomach, if the temperature is high.

dog ran 50 minutes of each hour, putting out in each of these experiments 142 kg.m. of energy per minute while running.

Under these conditions the best performance recorded in Fig. 3 when working without fuel was a run of $6\frac{1}{2}$ hours to complete exhaustion. Four days before this, when fuel was supplied, the same dog ran for 13 hours and was not exhausted at the end. The room temperature was 15° C. in each case, the rectal temperature remained between 39° and 40° C. and blood lactic acid concentration remained near the resting level. There is every reason to believe that exhaustion came from depleted fuel in the $6\frac{1}{2}$ -hour run and that the administration of easily available fuel in the 13-hour run resulted in twice the output of energy without exhaustion at the end and with the blood-sugar level maintained at or above the resting level. The actual energy outputs in these two cases were 46,150 kg.m. and 92,300 kg.m. per kg. of body weight.

PROTOCOL I. Record of a 17-hour run.

Energy output, 176 kg.m. per minute or a total of 150,000 kg.m.; a 5-minute rest period each half-hour was used for making observations and supplying water and fuel.

Time (hr.)	Remarks	Temperature		Intake		Blood sugar mg./100 c.c.
		Room ($^{\circ}$ C.)	Rectal ($^{\circ}$ C.)	Water (c.c.)	Fuel (g.)	
0	Start	15.5	38.7	—	—	—
0-1	—	15.0	39.5	340	40	—
1-2	—	14.7	39.5	250	40	112
2-3	—	15.5	39.6	180	40	131
3-4	—	15.8	39.7	330	40	—
4-5	Defecated	16.0	39.6	270	40	127
5-6	Refused candy	15.6	39.9	270	0	132
6-7	Urinated	14.8	39.8	120	40	—
7-8	Defecated	14.8	39.8	30	40	120
8-9	—	14.0	39.7	180	20	111
9-10	—	12.9	39.6	50	20	—
10-11	—	15.7	40.0	105	20	107
11-12	Refused candy	14.4	39.6	215	0	—
12-13	Refused candy	13.6	40.0	100	0	103
13-14	Glucose by stomach tube	13.6	39.8	295	42	—
14-15	Glucose by stomach tube	13.4	39.7	355	50	94
15-16	Refused candy	14.0	39.7	100	0	—
16-17	Tired but not exhausted	13.7	39.5	100	0	101

Three later experiments, not illustrated in Fig. 3, may be referred to briefly. With the treadmill running at the same rate, our second dog, with fuel supplied, ran for 9 hours. By that time she was becoming tired and her temperature had risen to 41.3° C. At this time the rate was decreased to 92 metres per minute, with the result that rectal temperature soon returned to normal and she was able to run easily for 15 more hours. At the end of 24 hours she was not exhausted notwithstanding an energy output

of 64,000 kg.m. during the first 9 hours and 79,000 kg.m. during the succeeding 15 hours, a total of 143,000 kg.m.

In the next experiment, detailed in Protocol I, Joe was used as a subject with the rate of work output one-fourth greater than in the experiments of Fig. 3. This higher rate of work output, 176 kg.m. per minute, is approximately the same as that which Rice and Steinhäus[1931] used. Under their experimental conditions, "rarely can a dog run continuously for 30 minutes." Joe had already maintained a still higher work output for 112 consecutive minutes (as already stated), and in this case he ran for 17 hours with fuel supplied and was not exhausted at the end. The total work output was 150,000 kg.m.

In the final experiment conditions were the same except that no fuel was supplied until after $4\frac{1}{2}$ hours' work. As may be seen in Protocol II,

PROTOCOL II. Record of a run demonstrating the effects of withholding and supplying fuel.

Conditions as in Protocol I except that food had been withheld for 36 hours and none was supplied until the onset of exhaustion. Then (during a rest period of 8 minutes instead of 5) 40 g. of candy restored the animal to an active state and simultaneously brought the blood sugar to its normal level. The experiment was discontinued when it appeared that the dog had returned to a steady state.

Time (hr.)	Remarks	Temperature		Intake		Blood sugar mg./100 c.c.
		Room (°C.)	Rectal (°C.)	Water (c.c.)	Fuel (g.)	
0	Start	13.5	38.5	—	—	78
0-1	—	14.8	39.8	120	0	88
1-2	—	16.6	40.0	120	0	82
2-3	—	16.0	39.7	140	0	71
3-4	Fan started	17.0	39.5	155	0	50
4-4 $\frac{1}{2}$	Exhausted	16.0	39.6	10	0	46
4 $\frac{1}{2}$ -4 $\frac{3}{4}$	Rest period; candy supplied	—	—	—	40	—
4 $\frac{3}{4}$ -5 $\frac{1}{4}$	Ran easily	15.8	39.8	420	20	127
5 $\frac{1}{4}$ -5 $\frac{3}{4}$	Ran easily	14.3	39.8	100	0	106

he was unable to run longer without fuel. He was revived by 40 g. of candy and was able to resume work within 8 minutes. The experiment was discontinued 1 $\frac{1}{2}$ hours later, for the dog seemed to be in good condition and able to continue for hours with fuel supplied.

A study of the reasons for exhaustion in these experiments naturally leads to a consideration of the heart rate. The importance of the cardi tachometer for this purpose is revealed by Fig. 4. The rapid decrease in heart rate during recovery shows that counts made in the usual way at the end of work may be 100 beats per minute too low. One rarely reads of a heart rate greater than 150 or 160 in the dog after work stops.

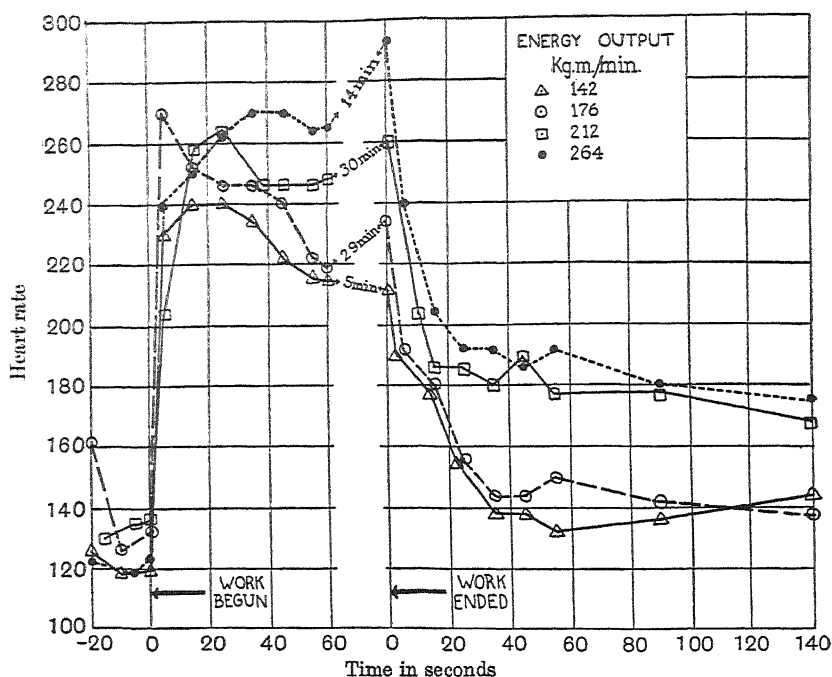


Fig. 4. Heart rate during work and recovery.

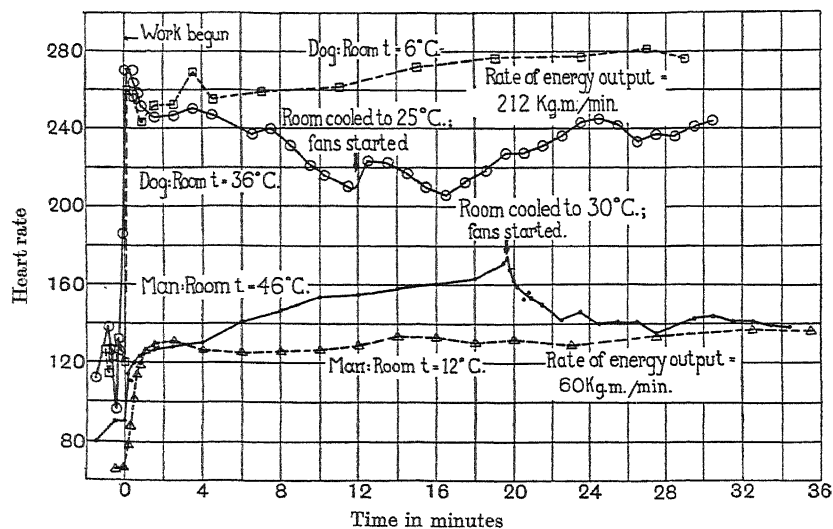


Fig. 5. The relation of heart rate to external temperature. With the onset of exhaustion during work in a hot room the heart rate of the dog decreases, that of man increases. As work continues after the room is cooled, the heart rate of the dog increases, that of man decreases.

Actually the rate after a steady state is reached is closely proportional to the rate of energy output and may be as high as 300 beats per minute.

It is not proposed at this time to present detailed observations on the heart rate during all these experiments. Two other sets of records will be presented briefly. The first of these, given in Fig. 5, shows the difference in response of man and dog to heat. As man approaches exhaustion at a high external temperature his heart rate rises and commonly the point of exhaustion coincides with the attainment of his maximum heart rate.

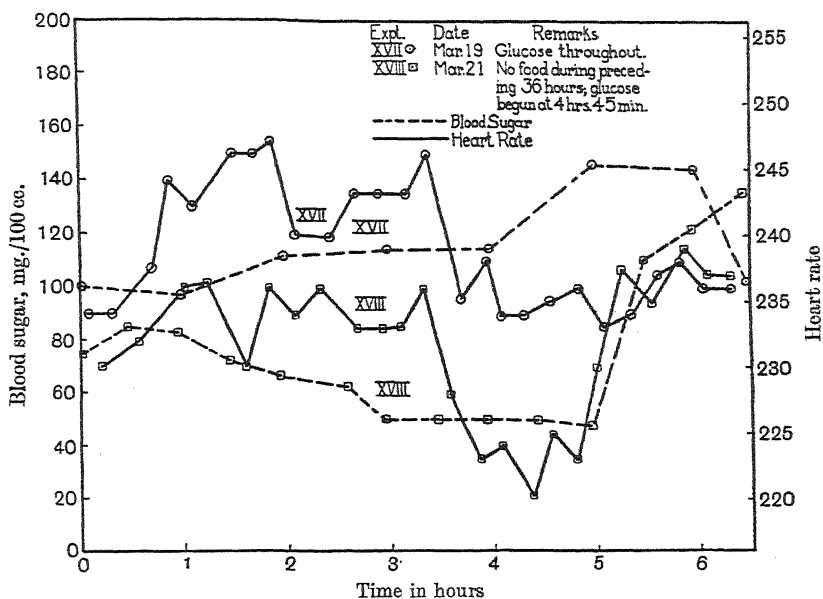


Fig. 6. Heart rate and blood sugar. Rest periods were interposed as described in the legend of Fig. 3.

In this case, fans were turned on the man and the room was cooled suddenly. Work was continued at the same rate, the heart rate decreased 30 to 40 beats per minute and within 3 minutes the subject had passed from a state of exhaustion to one of comfort. The dog, on the other hand, showed a decrease in rate with the onset of exhaustion, and after the room was cooled recovery (which was slower than in man) was accompanied by an increase in heart rate. Similar observations have been made on other occasions; evidently the control of the heart rate under these conditions is different in the two species.

Fig. 6 shows simultaneous observations of heart rate and blood sugar in two experiments. While there are large changes in heart rate which are

not parallel to the observed changes in blood sugar concentration, it is nevertheless a fact that in the experiment in which no fuel was given until the onset of exhaustion the minimum heart rate was reached shortly before candy was given. As the blood sugar increased from 50 to 100 mg./100 c.c., the heart rate increased from 220 to 235 beats per minute. Further experiments on this subject are planned.

The experimental results are of such a character that little discussion is necessary. The literature has been reviewed recently by Rice and Steinhaus [1931], by Campos, Cannon, Lundin and Walker [1929], and by Dische and Goldhammer [1932]. The experiments of Rice and Steinhaus indicated that the energy output of a dog in treadmill running might be limited by inadequate heart dissipation. Our experiments confirm this and show that by decreasing the external temperature the working capacity can be greatly increased. They were mistaken, however, in supposing that man possesses better facilities for heat regulation than the dog. The significant difference, at least in ordinary environmental conditions, is that the dog can produce heat at a much greater rate than man. We have found that an athlete running on a horizontal treadmill at the rate of 233 metres per minute with a room temperature of 22° C. and virtually no air movement could not dissipate heat quite as fast as its rate of production. At the end of 80 minutes he was exhausted; his rectal temperature was 40.6° C. His energy output as calculated from the oxygen intake and respiratory quotient was about 100 kg.m. per minute per kg. of body weight. The dog Joe was able to maintain a constant temperature with an energy output greater by at least 50 p.c. with the same environmental conditions. This represents a greater rate of energy output than can be attained by any athlete. We have made a comparison of the working capacity for short periods of this dog with that of a trained runner. Joe ran at the rate of 311 metres per minute on a grade of 17.6 p.c. for 7½ minutes to exhaustion. A mile runner, able at the time to run a mile in 4 minutes and 30 seconds, was exhausted with the treadmill at the same grade after 6 minutes at the rate of 125 metres per minute. Evidently this dog has a capacity for expending energy which is 2½ times greater than that of an athlete. It is not surprising, therefore, that heat dissipation is a more common limiting factor than with man¹.

The experiments of Eagle and Britton [1932] showed that the

¹ Dogs no doubt differ greatly in heat dissipating capacity. Perhaps Joe has a greater than average ability to dissipate heat on account of his small size and short hair. The dogs used by Rice and Steinhaus were twice as large and possibly were handicapped on this account.

administration of their cortico-adrenal extract to normal dogs increased their capacity for work. We are indebted to Dr Eagle for supplementary information regarding their experimental conditions. No water was given to their dogs during exercise and the room temperature was about 25° C. They report some decrease in blood sugar, although this was not simply related to degree of exhaustion. The total energy output before cortico-adrenal extract was about 15,000 kg.m. and from 20,000 to 25,000 after. The work output was at a lower rate, but even with cortico-adrenal extract the total output was only one-sixth as great as in our 17-hour experiment on Joe. The rate was the same and the total work output about two-fifths as great as in the 9-hour experiment on our second dog. The most reasonable interpretation of the effect on working capacity of cortico-adrenal extract is that it is indirect, possibly helping in mobilizing fuel, possibly eliminating unpleasant sensations arising from thirst, hunger and overheating. The same may be said of the similar effect of adrenaline on working capacity. In the most successful experiment of Campos, Cannon, Lundin and Walker [1929] in demonstrating the effect of adrenaline, the rate of work output was four-fifths as great and the total work output one-third as great as in our 17-hour experiment. Neither extraneous adrenaline nor cortico-adrenal extract *per se* is necessary even when the exercise is of long duration.

SUMMARY.

A study has been made of three factors which may limit the capacity of a dog for work—external temperature, supply of oxygen, and supply of fuel. A fourth factor perhaps of equal importance is the supply of water. The water intake has been observed in all these experiments, but the limiting effect of inadequate water intake has not been studied.

With external temperature low and oxygen supply adequate and when fuel and water are supplied, the performance of a dog is virtually tireless. In one case a dog ran for 17 hours with 5-minute rest periods each half-hour. In the 17 hours he ran 132 km. (82 miles) and climbed 23 km. (14 miles) with a total energy output of 150,000 kg.m. per kg. of body weight. His working capacity was increased more than three times by supplying fuel. His energy output in this run was three times greater than in any recorded experiment showing the influence of adrenaline injections on working capacity, and six times greater than in similar experiments with cortico-adrenal extract. Evidently the dog does not require an extraneous supply of either of these hormones for work of many hours' duration.

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PHYSIOLOGICAL LEUCOCYTOSIS.

II. Post-prandial leucocytosis and Widal's hæmoclastic test for hepatic efficiency.

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VARIOUS opinions have been expressed on post-prandial leucocytosis, and Widal's hæmoclastic test for hepatic efficiency.

Among recent authors, Beaumont and Dodds [1931] reported that "Widal's hæmoclastic reaction...is held to be quite reliable," while Rolleston and McNee [1929] deprecated the test and quoted Shaw's views that "the test lacks any theoretical basis, and is of no value in the diagnosis of hepatic efficiency." Further work was clearly necessary.

Before Shaw's investigation [1925] most workers confirmed Widal's work, although its clinical significance was uncertain. The existence of post-prandial leucocytosis is denied by Shaw, who suggested in a later paper [1927] that the observed leucocytosis was due to the normal diurnal variation of the leucocytes. While Shaw's work would appear to be based upon careful experimental work, it has not been repeated. Shaw's theory of the diurnal variation is not new. It had been advanced 27 years previously by Japha [1900], who however concluded that there was a post-prandial leucocytosis quite distinct from the diurnal variation.

METHOD.

In the present work the technique described in Part I [Martin, 1932] was used.

In order to make sure that the results obtained were due to the ingestion of protein and not to the normal variation in the leucocyte count, a series of control experiments was carried out on fasting and resting patients (Table I). Blood samples were taken at approximately the same intervals as in the subsequent experiments.

TABLE I. Control experiments. Variations in the leucocyte count of fasting subjects.

Time in min.	Leucocytes per c.mm.					
	<i>Case 1</i>	<i>Case 2</i>	<i>Case 3</i>	<i>Case 4</i>	<i>Case 5</i>	<i>Case 6</i>
0	8,400	9,000	7,400	6,600	6,400	7,700
25	8,200	9,300	6,700	7,800	6,200	7,300
50	8,600	8,800	6,700	6,800	6,800	7,200
100	8,600	9,200	6,200	7,800	6,500	7,200

I. THE EFFECTS OF A FULL MEAL ON THE LEUCOCYTE COUNT.

The effects of a full meal on the leucocyte count were investigated on six normal subjects and three cases of hepatic disorder. Of the latter, case 13 was one of mitral disease with enlarged liver and jaundice, and Nos. 14 and 15 were cases of multilobular hepatic cirrhosis with ascites. The patients were confined to bed and starved from the previous midnight. A meal of meat, potatoes, cabbage, milk pudding and half a pint of milk was given at 12.15 p.m. A sample of blood was taken immediately before the meal. Three more samples were taken at 20, 50 and approximately 110 min. after the meal. The results obtained are given in Table II.

TABLE II.

Normal subjects				Hepatic cases	
Min.	Leucocytes per c.mm.	Min.	Leucocytes per c.mm.	Min.	Leucocytes per c.mm.
<i>Case 7</i>		<i>Case 10</i>		<i>Case 13</i>	
	8,000		10,400		13,400
20	9,000	20	10,700	20	14,200
50	8,800	50	9,700	50	12,200
120	8,300	100	8,700	120	11,500
<i>Case 8</i>		<i>Case 11</i>		<i>Case 14</i>	
	8,400		6,200		6,000
20	9,300	20	6,300	20	5,200
50	9,200	50	6,200	50	6,800
110	9,500	100	6,400	100	7,500
<i>Case 9</i>		<i>Case 12</i>		<i>Case 15</i>	
	7,400		7,900		5,500
20	7,000	20	6,500	20	4,200
50	6,200	50	7,300	50	4,100
100	5,700	100	6,200	100	5,300

The leucocyte counts taken 20 min. after the meal yield a rise in four out of the six normal cases, and a fall in two of the three hepatic cases. The later counts are quite irregular. A comparison of Tables I and II shows that the leucocyte variations after the meal are similar in degree and sense to those obtained from the fasting subjects.

II. THE EFFECTS OF THE INGESTION OF MILK ON THE LEUCOCYTES OF THE BLOOD.

Widal and co-workers [1920] claimed that the ingestion of 8 oz. of milk would cause a leucocytosis in healthy persons and a leucopenia in patients with hepatic disorder. Both Wilson [1922] and Feinblatt [1923] confirmed this on a large number of patients.

This test was carried out on six normal subjects (Nos. 16-21), one case of syphilitic cirrhosis of the liver with ascites (No. 22), one of carcinoma of the gall bladder with hepatic metastases and jaundice (No. 23), and one of multilobular cirrhosis with ascites (No. 24). The patients were starved overnight and confined to bed on the day of the test. Ten ounces of milk were given about 10.0 a.m. Samples of blood were taken just before giving the milk and at approximately 25, 50 and 80 min. afterwards.

TABLE III.

Normal cases				Hepatic cases	
Min.	Leucocytes per c.mm.	Min.	Leucocytes per c.mm.	Min.	Leucocytes per c.mm.
<i>Case 16</i>		<i>Case 19</i>		<i>Case 22</i>	
	7,000		6,100		12,500
25	7,300	25	6,800	25	12,300
50	7,500	50	6,300	65	10,700
85	6,000	75	6,800	100	12,500
<i>Case 17</i>		<i>Case 20</i>		<i>Case 23</i>	
	7,400		7,800		7,800
25	7,600	25	10,000	25	7,600
75	6,300	50	8,200	85	7,700
120	7,600	80	9,000	120	9,300
<i>Case 18</i>		<i>Case 21</i>		<i>Case 24</i>	
	7,700		6,400		9,600
25	7,900	25	6,900	25	10,100
50	6,100	50	8,000	50	10,800
80	6,200	90	8,900	80	10,900

In all the normal subjects there is an increase of leucocytes during the first 25 min., and in the hepatic cases there is a corresponding diminution in two out of the three cases. The 50 and 80 min. readings are quite irregular and it is not possible to draw any conclusions from them. In all the above cases the variations lie within the limits of the normal resting patient.

III. THE EFFECT OF THE INGESTION OF PEPTONE ON THE LEUCOCYTE CONTENT OF THE BLOOD.

Most of the investigators of post-prandial leucocytosis, including Widal and co-workers [1920] and Schiff and Stranski [1921], attributed the phenomena to protein degradation products. The results just tabulated fell within the range of the fasting patient. If products of protein digestion could be given in large amounts, it was hoped that the post-prandial leucocytosis could be so increased that the counts obtained would lie outside the normal range. Peptone was therefore used, since it is non-toxic and can be given in large quantities. Six normal persons (Nos. 25-30), one case (No. 31) of syphilitic hepatic cirrhosis with jaundice, one case (No. 32) of jaundice due to malignant nodules in the liver, and one case (No. 33) of advanced multilobular portal cirrhosis with ascites, were examined. They were starved overnight. A blood sample was taken and $1\frac{1}{2}$ oz. of Fairchild's peptone, dissolved in the minimum amount of water ($1\frac{1}{2}$ oz.), were administered. Blood counts were made at 30, 60 and 100 min. afterwards (Table IV).

TABLE IV.

Normal cases				Hepatic cases	
Min.	Leucocytes per c.mm.	Min.	Leucocytes per c.mm.	Min.	Leucocytes per c.mm.
<i>Case 25</i>		<i>Case 28</i>		<i>Case 31</i>	
	7,900		11,900		3,400
20	9,000	30	12,800	30	3,300
50	8,600	60	11,900	60	3,500
80	8,100	90	12,300	85	3,000
<i>Case 26</i>		<i>Case 29</i>		<i>Case 32</i>	
	9,000		9,200		12,300
20	10,100	30	7,500	30	12,900
50	9,900	60	9,100	60	14,000
75	9,900	120	11,700	85	12,000
<i>Case 27</i>		<i>Case 30</i>		<i>Case 33</i>	
	8,500		6,600		6,800
20	9,000	30	7,000	30	7,400
50	9,000	60	7,000	60	7,000
70	10,000	100	8,000	80	4,000

All the normal subjects, except No. 29, show a rise after the ingestion of peptone, which is most marked at the end of the first half-hour. Of the hepatic cases one showed a fall during the first half-hour and two showed a rise. As in the experiments on the effects of a full meal and of milk the variations are of the same degree as in the fasting patient.

SUMMARY.

The effect of giving a full meal, milk and peptone to normal subjects and patients with advanced hepatic disorder has been studied. The results fell within the normal range of the leucocyte count of the fasting subject.

I should like to thank Prof. F. E. Tylecote for his encouragement and help, Dr F. H. Smirk for suggestions and Miss M. J. Rickards, B.Sc., for help with the counts.

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THE ACTION OF ADRENALINE UPON THE CARDIAC VAGUS CENTRES.

BY G. STELLA.

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It has been known, since Oliver and Schäfer's experiments [1894], that adrenaline injected intravenously causes a rise of arterial pressure together with slowing, often considerable, of the heart rate. The latter is brought about through vagal inhibition, since it is no longer observed, after cutting both vagi centrally to the heart. As to the direct causes of this excitation of the inhibitory cardiac centres, opinion is still divided: some authors think that it is due to a direct effect of the drug upon the cardio-inhibitory centres, others explain the slowing simply as a reflex response to the rise of arterial pressure. The latter view is held, among recent workers, by C. Heymans, as will be seen below [1929, 1930]. Biedl and Reiner [1898] injected adrenaline into the peripheral end of a carotid artery and noticed an immediate effect upon the heart rate, which was slowed down to a considerable extent: this effect, however, was of very short duration: the rate soon recovered, to slow down again in the usual manner coincidently with the rise in general blood-pressure. They concluded that the second slowing of the heart represented a reflex from arterial hypertension, and that this was the mechanism of bradycardia in the case of intravenous injection. Also Gerhardt [1900] pointed out that adrenaline bradycardia usually appears only after the arterial pressure has risen to a certain degree, and believed the latter to be the only cause of the former. In some experiments, however, in which very small doses of adrenaline had been used he observed a well-marked heart slowing not accompanied by any rise of general blood-pressure.

It seemed difficult, however, to distinguish between a direct and an indirect action of adrenaline in these experiments: even in the case observed by Gerhardt of bradycardia without hypertension, the cause might have been a more abrupt rise of the arterial pressure at each

systole, as a consequence of the adrenaline acting directly upon the heart: whereas, on the other hand, the usual coincidence of bradycardia with hypertension by no means excludes a concurrent central effect.

Anrep and Starling [1925] studied the problem on dogs with the head perfused by means of a heart-lung preparation. They found that adrenaline injected into the separate head circuit and thus incapable of directly affecting the aortic pressure produced a well-pronounced diminution of the heart rate coincident in time with the adrenaline bradycardia, which follows an intravenous injection in the whole animal.

The same results were obtained by Anrep and Segall [1926], using the technique of the innervated heart-lung preparation, in which the systemic blood-pressure was kept under control and constant throughout the action of adrenaline.

The head circuit, in the experiments of Anrep and Starling, and of Anrep and Segall, however, included the carotid sinus with all its nervous connections intact. Now during the action of adrenaline the peripheral vaso-constriction in the head caused a rise of carotid pressure; this, however, was not the only cause of the observed bradycardia as was shown by the fact that the latter was still produced, although to a less extent, when care was taken to prevent the rise of carotid pressure and keep it constant throughout. Moreover, Volhard [1930], who repeated Anrep and Segall's experiments on dogs with denervated carotid sinus, obtained the same results, *i.e.* that adrenaline causes slowing of the heart, independently of increase of pressure in the territories of the depressors and carotid sinus nerves: this view of adrenaline bradycardia independent of peripheral hypertension is opposed by C. Heymans: as a crucial test to this thesis he points out [1930] that if both depressor and carotid sinus nerves be previously cut, adrenaline in the usual doses completely fails to cause any slowing of the heart, in spite of the fact that both vagi are left intact and in good condition.

The reason for the difference between his results and those of Volhard is to be sought, according to Heymans, in the different conditions under which the head was kept in the two cases.

METHOD AND EXPERIMENTAL RESULTS.

The experiments of Heymans, although they may be accepted as showing that adrenaline does not actually excite the cardio-inhibitory centres, cannot be taken as a proof that it has no effect on these. It is still possible, for instance, that this substance increases the reflex

excitability of the vagus centres, and that the fundamental difference between Heymans's and Volhard's techniques is not the perfusion of the head in the latter's with defibrinated blood, but rather the fact that the afferent nerves for the reflex regulation of the heart rate had been left intact in one case [Volhard] and cut in the other.

In the present work the effect of adrenaline upon the degree of reflex slowing of the heart, elicited by a rise of pressure in the region of the carotid sinus, or in the territory of the depressor nerves was investigated.

(1) ADRENALINE AND CARDIAC REFLEXES FROM THE CAROTID SINUS.

Dogs were used throughout, anaesthetized with morphine (0.5 cg. per kg.) and urethane (0.30 g. per kg.). One or both carotid arteries at the region of the carotid sinus were perfused with defibrinated blood: for this purpose cannulae were placed one in the common carotid about 2 cm. below the bifurcation, for the inlet of the perfusion fluid, a second in the external carotid, distal to the origin of the lingual artery, for the outlet, and a third in the lingual artery and connected to a mercury manometer—all other arterial branches leaving the carotid between the cannulae having been previously tied, including all nervous connections, with the exception of the nerve of Hering. For the perfusion, a Dixon pump with a variable resistance in series was employed, as already described [G. Stella, 1931].

When only one sinus was perfused, the other was denervated: also the depressor nerves were carefully cut on both sides, just below the origin of the laryngeal superior [Koch's technique, 1931]. When the last operation was successful it was found that, if adrenaline was injected, while the pressure in the carotid sinus was maintained constant and low (40–50 mm. Hg), the resulting rise in general arterial pressure was not accompanied by any cardiac slowing. That this negative result was not due to an accidental destruction of the efferent cardiac fibres in the vagus trunk was proved by subsequently raising the pressure in the perfused carotid sinus, when the usual effect of slowing of the heart rate was produced. The injection of adrenaline, while the carotid sinus pressure remained low and constant, was thus used at the beginning of each experiment as a routine test for checking the complete destruction of the depressor fibres running with the vagus; the same test was also carried out at the end of the experiment to make sure that no recovery of damaged depressor fibres had meanwhile taken place.

The absence of cardiac slowing from adrenaline, under the conditions

above described, is in agreement with Heymans's [1930] findings, *i.e.* absence of cardiac inhibition from adrenaline after denervation of the carotid sinuses and section of the depressor nerves: in fact when the pressure in the carotid sinus is maintained at 40–50 mm. Hg and not pulsating, the depressor impulses from the sinus may be considered to be practically cut off [Heymans and Bouckaert, 1930; Stella, 1931;

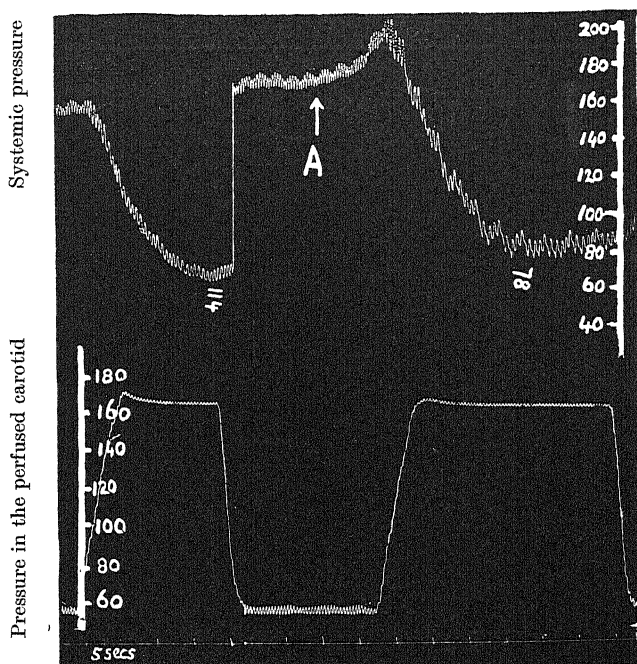


Fig. 1. Dog 11 kg. With both depressors cut and left carotid sinus denervated. Right carotid sinus perfused. Upper tracing: pressure in the femoral artery (mercury manometer). Lower tracing: pressure in the perfused sinus (manometer in the lingual artery). At A 1 c.c. of 1 : 40,000 adrenaline was injected into the femoral vein.

Bronk and Stella, 1932]. On the other hand, it was found that if, soon after the intravenous injection of adrenaline, the pressure in the perfused carotid sinus was raised to such a level as would usually cause only a slight slowing of the heart rate, the degree of slowing was definitely greater and sometimes considerably greater.

Fig. 1 shows one such experiment: here only the right carotid sinus was perfused, and the left denervated. It will be seen that a rise of the perfusion pressure from about 60 mm. Hg to 165 mm. before and after

injection of 1 c.c. of 1 : 40,000 of adrenaline (at *A*) has a quantitatively different effect upon the heart rate.

Again, if adrenaline is injected while the pressure in the sinus is high, a further retardation of rate is seen to occur, as shown in Fig. 2.

In other cases the effect was much more pronounced, and this was especially so when both carotid sinuses were perfused: thus in the instance reproduced in Fig. 3 *a* and *b*, the difference in the slowing of

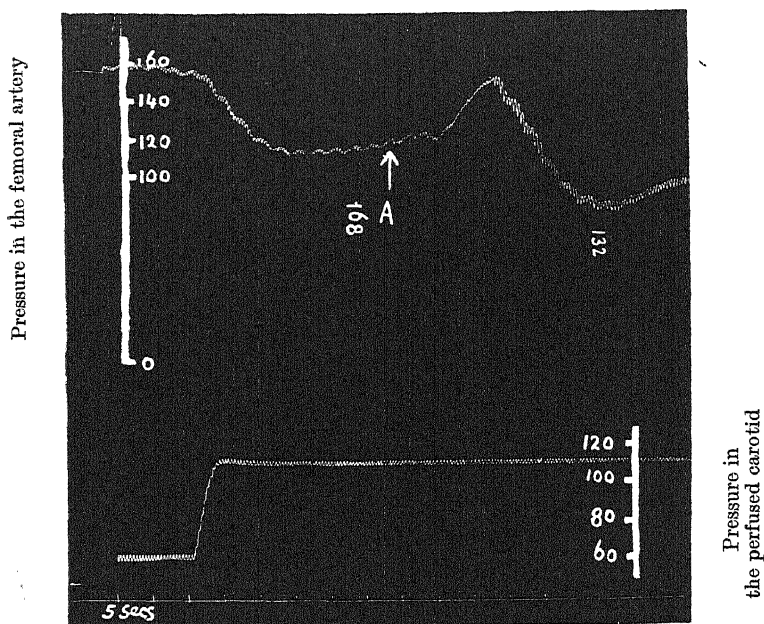


Fig. 2. Dog 10.5 kg. Conditions same as in Fig. 1. At *A* injection of 1.1 c.c. of 1 : 40,000 adrenaline in femoral vein.

the heart before and after adrenaline almost reaches 300 p.c. Fig. 3 *b* is also of interest because it shows the fact already described, *i.e.* that adrenaline under these conditions had no effect upon the heart rate until the pressure in the sinus was raised.

Two obvious possibilities presented themselves as to the causes for the observed results, *i.e.* either an action of adrenaline upon the cardio-inhibitory centres, making them more excitable to afferent stimuli, or else a peripheral action making the heart more responsive to vagal inhibitory impulses, or both. A hint as to a possible peripheral action of adrenaline is given by the work of M. Kuroda and Y. Kuno [1915] who

found, in the dog heart-lung preparation, that after the addition to the blood reservoir of 0.03–0.05 mg. of adrenaline stimulation of the vagus was as effective as, or even more effective than before. Before them X. Mathieu [1904] found that in dogs with both vagi cut, and in which one vagus was continuously stimulated, the injection of 0.5 mg. adrenaline caused a further slowing of the heart. The doses used by the latter

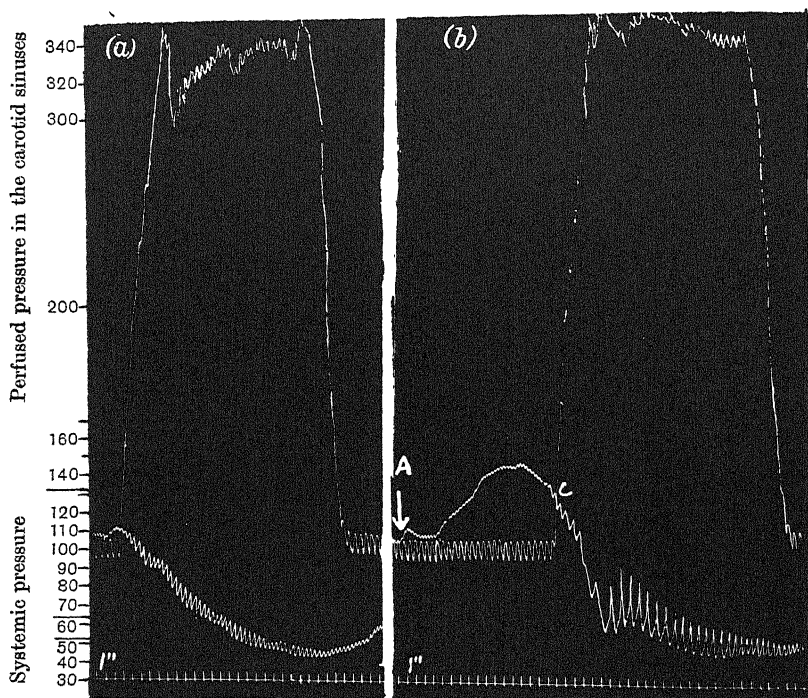


Fig. 3 *a* and *b*. Dog. 13 kg. Both depressors cut. Both carotid sinuses perfused. At *A* intravenous injection of 0.5 c.c. of 1 : 10,000 adrenaline.

author are, however, far above those employed in the present experiments, while the experimental conditions obtained by Kuroda and Kuno are not directly comparable with the present ones; therefore a number of experiments were performed on dogs anaesthetized in the manner already described and with both carotid sinus nerves and vagi cut; one vagus or the other was then stimulated faradically for short times and at regular intervals: it was found that adrenaline injected into the femoral vein in the amounts equal to those employed in the previous experiments did not cause any measurable change in the degree of heart

inhibition from vagus stimulation. It seems, therefore, that the increase of reflex inhibition of the heart described above must be due to a central action of adrenaline, produced either directly or indirectly, perhaps through vascular changes in the brain. This conclusion is further supported by experiments described below in which adrenaline was made to

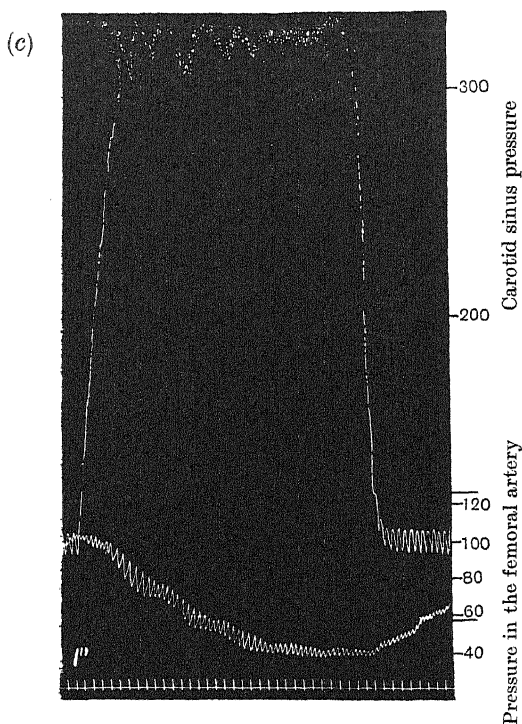


Fig. 3 c. Same as Fig. 3 a and b. The perfusion pressure is raised again, after 2 min. from *A* (see Fig. 3 b). It shows that the effect upon the heart rate is only slightly bigger than before the injection (Fig. 3 a).

circulate through the head, but prevented from coming into contact with the heart.

It will be seen in Fig. 2 that the first effect of adrenaline in causing the reflex slowing of the heart rate appears after a certain interval of time after the injection, and coincides with a moment at which the arterial pressure has risen to some extent. This same interval is seen in the intact animal. It appears, therefore, that in the latter bradycardia does not set in only as a result of an appropriate rise in arterial pressure,

but also because at that moment the adrenaline is displaying its central effect.

In the intact animal the degree of adrenaline bradycardia is great at first, and diminishes subsequently: this again, on the basis of the present experiments, must be interpreted mainly as a consequence of the subsidence of the action of adrenaline upon the cardiac centres: in fact, if the pressure in the carotid sinus be raised at different times after the end of the injection of adrenaline it is found that the slowing is of very different degrees, great at first, and then smaller and smaller. Fig. 3 c, for instance, shows that the effect of adrenaline is nearly over after 2 min. from the injection.

(2) REFLEX SLOWING OF THE HEART FROM RISE OF PRESSURE IN THE TERRITORY OF THE DEPRESSOR NERVES.

These experiments were performed on the innervated heart-lung preparation of dogs [Anrep and Segall, 1926]. The head was perfused with defibrinated blood by means of a Dixon pump, through the brachiocephalic artery; the blood from the reservoir going partly into the inferior vena cava and heart-lung, partly to the pump and head. After circulating through the head the blood came back to the heart through the superior cava and through the lungs back to the reservoir. Previous to the establishment of the artificial circulation, the carotid sinus on both sides was carefully denervated. The vagi and depressor nerves were instead left intact.

The experimental conditions thus obtained allowed a complete control of pressure and of the rate of circulation in the head and in the arch of the aorta. It was thus possible to vary the pressure in the aorta within very wide limits, while keeping that in the head practically constant. In complete agreement with the results described in the first part of this paper, it was found here, too, that the reflex slowing from hypertension in the left heart and aorta was always more marked while adrenaline was circulating in the head. Under the conditions described above the adrenaline after perfusing the head was returned to the heart. To exclude completely the possibility of a peripheral effect as the cause of the result, a number of experiments was carried out, in which the venous blood returning from the head was temporarily not allowed to enter the heart-lung circuit, but was instead collected into a separate reservoir. To this purpose a cannula was placed in the right subclavian vein, near its origin from the superior cava: by clamping the latter below

the cannula, the venous blood passed into the cannula and through a rubber tube into a beaker kept 10-15 cm. below the level of the heart. This deflection of the course of the blood from the head was only maintained long enough to allow a comparison between the effect of aortic pressure before and after adrenaline.

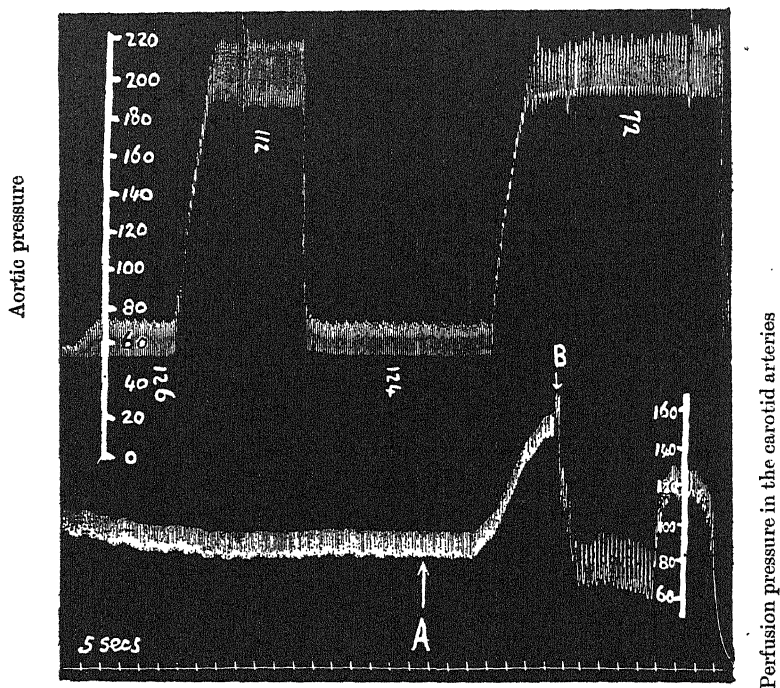


Fig. 4. Dog. 12 kg. Innervated heart-lung preparation. Head (lower tracing) perfused by means of a Dixon pump. At *A* 0.8 c.c. of 1 : 40,000 adrenaline is slowly injected in the tube leading to the pump and head. At *B* the output of the pump perfusing the head is diminished and brought down below the level it had before the injection. Upper tracing: pressure in the aorta. Lower tracing: pressure in the carotids.

Figs. 4 and 5 represent two such experiments. In both, at *A*, a small dose of adrenaline was slowly injected by means of a syringe into the tube leading to the Dixon pump and head. The increase of pressure in the head (see lower tracing) caused by the vaso-constriction from adrenaline was counteracted at *B* by diminishing the output of the pump.

The upper tracing is the pressure record in the aorta; both in the case of Fig. 4 and that of Fig. 5 the effect of adrenaline upon the reflex

slowing of the heart from aortic rise of pressure is well pronounced: in Fig. 4 the difference is of the order of 1-1.5, in Fig. 5 it is of the order of 1-2, these figures representing the usual range in the majority of all the experiments performed.

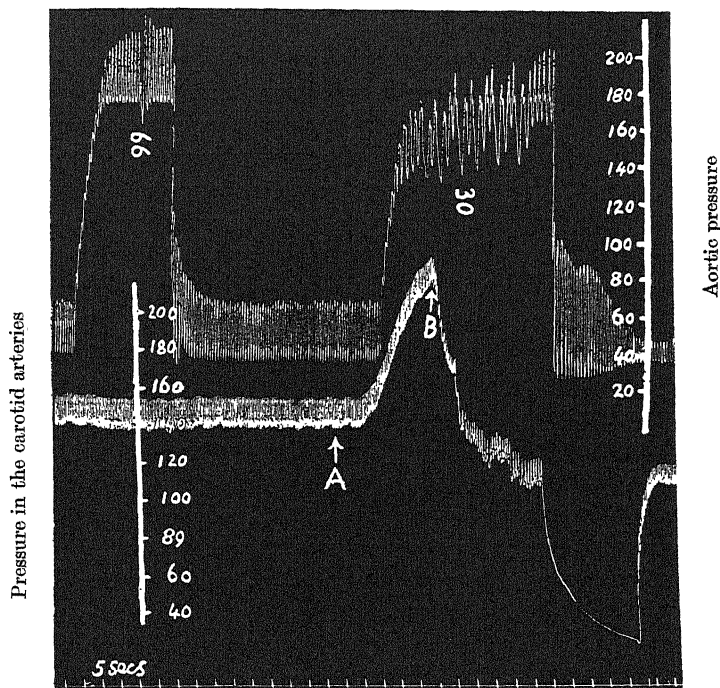


Fig. 5. Dog, 10 kg. Same as in Fig. 4. At A 1 c.c. of 1 : 50,000 adrenaline injected into the head circuit. At B perfusion pressure in the head artificially lowered.

CONCLUSION.

It has already been pointed out that the increased reflex response of the heart rate to pressure changes during the action of adrenaline cannot be explained as a result of peripheral sensitization to inhibitory vagus impulses; the experimental conditions employed, moreover, exclude an action upon the peripheral end of the afferent path of the reflex, so that the most likely possibility is that the cardio-inhibitory centres, under the action of adrenaline, become more excitable. This conclusion is not in agreement with that arrived at by C. Heymans [1929] according to whom "*l'adrénaline n'a aucune action spécifique, ni directe,*

ni indirecte, sur le centre cardio-inhibiteur du chien"; but it would seem that the experimental conditions employed by Heymans (section of the afferent path of the reflex) were not such as to justify that conclusion. The only justifiable conclusion to be drawn from them would be that adrenaline does not actually excite the cardiac vagus centre. The doses used by Heymans, and in the present series of experiments, were very moderate ones, just such as would give in the intact animal a good rise of arterial pressure together with definite slowing of the heart: whether doses much higher than those might excite the vagus centres directly, or better, independently of afferent inhibitory impulses from the depressor and carotid sinus nerves, was not studied.

What is the mechanism through which adrenaline increases the reflex responses of the cardiac centres of the vagus to afferent stimuli? Before entering the discussion of this problem, which anyhow must be left open at the present, it is interesting to note that the results of the present work seem to support a view put forward by G. Viale [1928, 1930], according to whom the vagal tone is maintained by the presence of adrenaline in the circulating blood. On the other hand, the fact that adrenaline does not directly excite the vagus centres, but simply makes them more responsive to afferent inhibitory impulses, perhaps explains the reason why the injection of adrenaline in an animal with otherwise very low arterial pressure and quick heart rate (low vagal tone) is not so effective in bringing down the heart rate [W. W. Sawitsch and E. N. Speranskaja-Stepanova, 1927].

According to Anrep and Starling [1925] the effect, upon the cardiac centres, of adrenaline circulating in the head is most probably an indirect one, brought about by changes in the calibre of the vessels in the brain and readjustment of flow and pressure in the different centres. They pointed out in fact that, even if the pressure in the carotids be kept artificially constant, during the action of adrenaline, the pressure in the Circle of Willis and the arteries originating therefrom must be higher than before adrenaline, owing to the peripheral vaso-constriction and slower rate of flow.

It is very unlikely, however, that the variations of pressure in the cerebral arteries are at any rate the only mechanism through which the described effect upon the heart rate is produced, the more so, since, according to experiments of Hering [1927] and Heymans [1930] differences of pressure also of very large magnitude in the cerebral circulation leave the heart rate unaffected, whereas the variations of pressure produced by adrenaline, when the carotid pressure is main-

tained constant, are bound to be of moderate degree. In the experiments described in the first part of this paper, the arterial pressure, at the very time during which adrenaline was displaying its action upon the cardiac vagal centres, was usually low, 80 mm. Hg, or lower, and it is difficult to conceive that it was of such importance for the heart rate. But things are still clearer in experiments such as those of Figs. 4 and 5, in which the cerebral pressure was artificially lowered well below the level it had before the injection of adrenaline, and in which bradycardia still remained. It appears, therefore, that variations of pressure in the Circle of Willis and its branches cannot represent the whole mechanism, and it seems right to say the whole mechanism because I found, in confirmation of Volhard [1930], that if the pressure in the brain circulation is not interfered with but allowed to rise under the action of adrenaline, the slowing of the heart becomes more marked still. Whether here the amount of blood and oxygen supplied at different perfusion pressures plays any part, or whether the effect is due to some sort of sensitization to changes of pressure, which might take place in territories, otherwise irresponsive to the latter, is a question for further study. Also the possibility of increased intracranial pressure cannot be dismissed.

SUMMARY.

1. Adrenaline, injected intravenously in dogs, in moderate doses (about 0.002–0.005 mg. per kg.), enough to cause increase of arterial pressure and marked slowing of the heart, does not seem to have any direct exciting effect upon the cardio-inhibitory centres.
2. Adrenaline administered in these amounts is, nevertheless, not entirely without action on the centres, since it increases the reflex excitability of the cardio-inhibitory centres to afferent impulses from the depressor and the carotid sinus nerves.
3. Adrenaline bradycardia is reflex in origin, and due to the arterial pressure acting upon the sensitive regions of the carotid sinus and of the territory of distribution of the depressor nerves: its degree, however, is conditioned by a state of increased reflex excitability of the cardiac vagus centres caused by the injected adrenaline.

In conclusion I want to thank Prof. C. Lovatt Evans for his advice and criticism. This work was begun at Cambridge during the tenure of the G. H. Lewes Studentship. For the part performed at University College the expenses were defrayed by a grant from the Foulerton Reserve Fund of the Royal Society, for which I am most thankful.

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PHLORRHIZIN DIABETES IN FASTING OR FED HYPOPHYSECTOMIZED DOGS.

BY A. BIASOTTI AND B. A. HOUSSAY.

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WE have shown that extirpation of the pituitary gland or of its anterior lobe, or cauterization of the tuber cinereum, prevents or diminishes diabetes following pancreatectomy in the toad. The implantation of the anterior lobe (and to a lesser degree the posterior) causes the onset of a severe diabetes in these operated animals [Houssay and Biasotti, 1931 *a*].

Also in the hypophysectomized toad, or toad without the anterior lobe, phlorrhizin glycosuria is less marked than in the control, the implantation of the anterior lobe causing it to increase [Di Benedetto, 1931].

In hypophysectomized dogs total pancreatectomy usually produces only a mild diabetes as compared with the controls, survival is prolonged (up to 6 months), the weight decreases more slowly, the hyperglycæmia and glycosuria are less, during fasting there is no great destruction of protein, the D : N ratio is low (0.7–1.85) and the basal metabolism is not raised or is raised only very slightly. In dogs with a lesion of the tuber cinereum pancreatic diabetes has the usual rapid development with marked glycosuria [Houssay and Biasotti, 1931 *c*]. The same occurs in thyroidectomized dogs [Yriart, 1930].

Phlorrhizin diabetes in fasting hypophysectomized dogs is characterized by death usually with hypoglycæmia, the glycosuria being only a third of that in the controls, the urinary nitrogen less, and the D : N ratio low. On the other hand, five dogs with lesions of the tuber cinereum had a phlorrhizin diabetes similar to that of the controls [Houssay and Biasotti, 1931 *b*].

A brief summary of the marked metabolic changes occurring in hypophysectomized animals may be found in our previous publications [Houssay, 1931].

METHODS.

We have made comparative studies of phlorrhizin diabetes during fasting in thirty-one dogs, seven hypophysectomized, four with the

posterior lobe removed, nine with lesions of the tuber cinereum, six thyroidectomized, and five controls. We have also compared the action of phlorrhizin in four hypophysectomized dogs and four controls on a meat diet, five hypophysectomized and four controls on a sugar diet, and four hypophysectomized and three controls on a fat diet.

Before the experiments the dogs were kept in metabolism cages on a diet of 400–500 g. of meat and the urine was measured daily. After a day of fasting they received intravenously 1 g. of phlorrhizin dissolved in 25 c.c. of 1.2 p.c. sodium bicarbonate solution; on subsequent days 1 g. suspended in 10 c.c. of olive oil was injected under the skin of the flank or abdomen. Kahlbaum's and Grüber's preparations of phlorrhizin were used. Each day the dogs were weighed, the volume of urine measured and determinations made of the urinary glucose (Benedict's method), nitrogen (Folin) and total ketone bodies (van Slyke). The blood sugar was determined (Hagedorn and Jensen) at the beginning and end of the experiment and sometimes every other day.

The fasting dogs could drink water *ad lib*. Those on a meat diet had 300 g. daily of good fresh lean beef, and those on a sugar diet 50 g. of cane sugar. Those on a fat diet received 100 c.c. of an emulsion of oil (600 c.c. of olive oil, 300 c.c. of water and 5 c.c. of 40 p.c. NaOH mixed together, and 48 hours later the emulsified oil decanted). The oil and at times the sugar was given by stomach tube.

The operations on the pituitary were performed by the left temporal route and verified at autopsy and by histological examination. The lesion of the tuber cinereum was done by galvano-cautery from the pituitary stalk to the posterior limit of the corpora mammillaria.

The starvation experiments lasted 5 days in thyroidectomized dogs and dogs deprived of the posterior lobe; in ten hypophysectomized dogs taken in the order in which they are presented in the tables 3, 5, 6, 6, 3, 3, 3 days respectively. They lasted 4, 3, 4, 4, 4, 6, 6, 6, 6 days in those with the tuber lesions and 7, 6, 5, 7, 3 days in the controls.

On meat diet the experiments lasted 7 days in the hypophysectomized dogs and controls; on sugar 6 days; on fat 5 days in the controls and in the hypophysectomized only 3, 3, 3 and 4 days owing to the onset of a severe or fatal hypoglycæmia.

The time elapsing after the operation and before the administration of the phlorrhizin was, in the order of the tables of this paper, in fasting experiments, after hypophysectomy 299, 260, 73, 194, 51, 120, 105 days; after lesions of the tuber cinereum 20, 26, 36, 47, 46, 45, 44, 44, 46 days; after thyroidectomy 55, 55, 55, 55, 137, 137 days; after removal of the

posterior lobe 97, 61, 57, 57 days; after the hypophysectomy for experiments on a meat diet 104, 51, 102, 342 days; on sugar 151, 136, 94, 143, 62 days; on fat 346, 35, 77, 74 days.

BLOOD-SUGAR RESULTS.

Hypophysectomized dogs readily showed severe hypoglycæmia under various conditions:

- (1) At times spontaneously.
- (2) Always during prolonged fasting.
- (3) Frequently after the injection of adrenaline during fasting.
- (4) During the action of insulin [Houssay and Magenta, 1925-7-9].
- (5) In some cases after total extirpation of the pancreas.
- (6) Always after injection of phlorrhizin [Houssay and Biasotti, 1931 b].

TABLE I. Dogs under phlorrhizin. Blood sugar mg./100 c.c.

Fasting.										
Hypophy- sectomized		Tuberal lesions		Without posterior lobe		Thyroid- ectomized		Controls		
Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final	
109	56	92	81	96	66	110	70	104	70	
106	70	108	118	84	98	118	90	110	114	
88	70	111	92	88	78	102	104	114	117	
106	72	120	95	82	66	120	80	124	118	
81	67	102	81			96	88			
		94	74			104	92			
		98	77							
		110	89							
Mean	98	67	104	88	87	77	108	87	113	104

Fed.												
Meat				Sugar				Fat				
Hypophy- sectomized		Control		Hypophy- sectomized		Control		Hypophy- sectomized		Control		
Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final	
113	104	92	84	102	90	96	104	88	57	114	127	
86	88	99	98	88	80	116	98	90	48	130	128	
118	94	104	90	80	86	98	106	91	58	92	106	
92	86	112	98	108	92	110	114	84	62			
				96	84							
Mean	102	93	101	92	94	86	105	105	88	56	112	120

In fifteen out of seventeen hypophysectomized dogs injected with phlorrhizin there was a severe or fatal hypoglycæmia. Some of these animals were saved by repeated intake of sugar and later of food, when the condition had not been present long. Of the fasting dogs injected

with phlorrhizin only the hypophysectomized showed intense hypoglycæmia and severe symptoms, several of them dying. These symptoms, appearing usually during the third or fourth day when the blood sugar had dropped to 0.07 p.c. were weakness, tremors, inability to stand, salivation, opisthotonus, stepping movements, partial or general convulsions, lowered temperature, slow respiration, flaccidity, and finally death. No such symptoms occurred during fasting in the controls, nor in any of the fasting animals which had had operations other than hypophysectomy.

Feeding with meat or sugar maintained the blood sugar normal and prevented symptoms in the hypophysectomized dogs. On a fat diet there was severe hypoglycæmia in the four hypophysectomized animals with death on the third or fourth day, but no hypoglycæmia occurred in the controls in 6 days.

Glycosuria.

During starvation the maximum elimination of sugar occurred on the third and fourth days, the D : N ratio being between 2.61 and 3.7 in the controls.

TABLE II. Dogs under phlorrhizin: glucose eliminated in g. per kg. per diem.
Average for each dog of each group.

Fasting.					
	Hypophy- sectomized	Tuberal lesion	Without posterior lobe	Thyroid- ectomized	Controls
	0.51	1.47	2.63	1.70	2.73
	0.70	1.15	2.87	2.45	1.84
	0.81	1.73	2.10	2.02	2.64
	0.91	0.90	1.64	2.00	1.25
	0.80	1.63		1.70	1.55
	0.42	1.70		1.61	
	0.66	1.70			
		1.92			
		1.51			
Mean	0.68	1.54	2.31	1.91	2.00

Fed.						
Meat		Sugar		Fat		
Hypophy- sectomized	Control	Hypophy- sectomized	Control	Hypophy- sectomized	Control	
3.61	4.02	1.98	5.08	0.57	2.07	
3.39	4.64	3.60	4.34	0.57	2.98	
3.20	3.91	2.67	3.19	1.04	2.62	
3.00	4.33	2.40	4.33	1.10		
		2.17				
Mean	3.30	4.22	2.56	4.23	0.82	2.55

As can be seen in Table II, among the fasting animals treated with phlorrhizin the controls, the thyroidectomized and those deprived of the posterior lobe showed marked glycosuria, while the elimination of sugar in those which had been hypophysectomized was only a third of that in the controls. In those with lesion of the tuber it was fairly high (1.54 g.) compared with that of the hypophysectomized (0.68 g.), but somewhat less than that of the controls (2.00 g.).

On meat and on sugar diets the daily glucose elimination per kg. in the hypophysectomized dogs treated with phlorrhizin was respectively 23 p.c. and 21 p.c. less than in the controls. On fat diet the elimination was comparable to that in fasting, *i.e.* the hypophysectomized eliminated 67 p.c. less sugar than the controls.

Diuresis was proportional to the quantity of sugar eliminated, the concentration of glucose being similar in all cases (5-8 p.c.). Nevertheless some of the dogs with lesion of the tuber cinereum showed polyuria dating from before the injections.

Urinary nitrogen elimination.

It can be seen from Table III that the nitrogen elimination in fasting dogs without phlorrhizin (7-10 days) was less in the hypophysectomized and thyroidectomized (0.25 g. per kg. per diem) than in the controls or

TABLE III. Urinary nitrogen in g. per kg. per diem. Average for each group.

	17 dogs hypophy- sectomized	5 dogs tuberal lesion	4 dogs thyroid- ectomized	10 dogs controls
Fasting without phlorrhizin	0.25	0.36	0.25	0.36
Fasting with phlorrhizin	0.36	0.66	0.63	0.80
Total increase	0.11	0.30	0.38	0.44
Increase p.c.	44	83	152	122

those with lesions of the tuber (0.36 g.). Phlorrhizin administered during fasting caused a very slight absolute increase in nitrogen elimination in the hypophysectomized (0.11 g.), a large increase in the controls (0.44 g.), slightly less in the thyroidectomized (0.38 g.), and less still in those with tuber lesion (0.30 g.). The proportional increase was larger in the thyroidectomized and controls, much less in the hypophysectomized and intermediate in those with lesions of the tuber.

On comparing the five groups of fasting dogs under phlorrhizin (Table IV), the greatest daily excretion of nitrogen per kg. occurred in dogs without the posterior lobe and in the controls, while in the hypo-

TABLE IV. Dogs under phlorrhizin: Urinary nitrogen in g. per kg. per diem.
Average for each dog of each group.

Fasting.					
	Hypophy- sectomized	Tuberal lesion	Without posterior lobe	Thyroid- ectomized	Control
	0.51	0.53	0.71	0.62	0.91
	0.33	0.44	1.24	0.75	0.78
	0.31	0.99	1.00	0.61	0.89
	0.34	0.68	0.95	0.60	0.52
	0.19	0.60		0.51	0.77
	0.21	0.52		0.51	
	0.62	0.69			
		0.60			
Mean	0.36	0.63	0.97	0.60	0.77

Fed.					
Meat		Sugar		Fat	
Hypophy- sectomized	Control	Hypophy- sectomized	Control	Hypophy- sectomized	Control
1.60	1.65	0.16	0.69	0.26	0.59
1.47	1.62	0.41	0.62	0.30	0.88
1.19	1.42	0.35	1.04	0.33	0.71
1.29	1.57	0.30	0.70	0.46	
		0.28			
Mean	1.37	1.56	0.30	0.76	0.73

physectomized it was much less (45 p.c. of that of the controls), and that in dogs with lesions of the tuber or excision of the thyroid it was intermediate.

On fat and sugar diets there was practically the same nitrogen elimination as in fasting, both in the hypophysectomized dogs and the controls. On a meat diet there was naturally a large increase, reaching 1.37 g. N per kg. daily in the hypophysectomized and 1.56 g. in the controls. As both groups received 9.1 g. N daily and the initial and final weights were 7.2 and 7.1 kg. in the hypophysectomized and 8.1 and 7.2 kg. in the controls it can be calculated that the first received 1.26 g. per kg. N daily and eliminated 1.38 g. and the controls received 1.12 g. and eliminated 1.56 g.; that is to say, the hypophysectomized lost 0.12 g. of their own nitrogen per kg. per diem and the controls 0.44 g., which explains in great part the decrease in weight in the latter. The meat given produced a certain saving of nitrogen in both groups, for if we add the amount eliminated during fasting to that given in the meat we get 1.91 g. for the controls and 1.62 g. for the hypophysectomized, whereas they actually eliminated 1.54 and 1.38 g. respectively, *i.e.* 0.37 and 0.24 g. less.

TABLE V. Initial weights (*a*), and final weights (*b*), of dogs treated with phlorrhizin.

Fasting.										
Hypophy- sectomized		With lesion of tuber		Without posterior lobe		Thyroid- ectomized		Controls		
<i>a</i>	<i>b</i>	<i>a</i>	<i>b</i>	<i>a</i>	<i>b</i>	<i>a</i>	<i>b</i>	<i>a</i>	<i>b</i>	
16	15.3	10.2	8.9	6.5	5.3	8.3	7.6	14.5	12.3	
17.9	16.0			11.2	9.0	7.1	6.1	10.7	8.8	
9.5	8.8	7.3	6.8	8.0	6.9	8.1	6.5	9.5	8.2	
9.1	8.5	6.3	5.0	7.6	7.0	9.5	8.4			
9.0	8.7	5.6	4.8			10.2	9.1	12.2	10.8	
10.8	10.1	9.0	8.2			7.4	7.1	9.4	8.0	
9.4	8.4	8.4	7.5							
		9.0	8.4							
		8.2	7.0							
Mean	11.7	10.8	8.0	7.0	8.3	7.0	8.4	7.5	11.2	9.6

Fed.										
Meat				Sugar				Fat		
Hypophy- sectomized		Control		Hypophy- sectomized		Control		Hypophy- sectomized		Control
<i>a</i>	<i>b</i>	<i>a</i>	<i>b</i>	<i>a</i>	<i>b</i>	<i>a</i>	<i>b</i>	<i>a</i>	<i>b</i>	<i>a</i> <i>b</i>
6.9	6.5	6.6	6.6	6.0	5.7	7.5	7.1	12.0	11.6	9.5 8.5
6.1	6.2	8.3	7.3	6.2	5.5	13.6	12.0	6.8	6.6	8.2 7.1
8.8	8.5	10.2	9.0	13.8	12.3	8.0	7.0	6.8	6.7	9.0 7.8
9.3	9.4	7.3	6.8	9.4	8.1			6.5	6.0	
				11.5	10.7					
Mean	7.7	7.6	8.1	7.4	9.3	8.4	9.7	8.7	8.0	7.7 8.9 7.8

Weight.

The administration of phlorrhizin during fasting caused a smaller loss of weight in the hypophysectomized (11 p.c.) than in the controls or in those with lesions of the tuber (15–16 p.c.), and the loss was intermediate in the thyroidectomized (13 p.c.). This difference was maintained on a sugar or fat diet; on meat diet the loss in the hypophysectomized was appreciably less than in the controls, in which the loss was less (11 p.c.) than during fasting. Apparently the loss of weight is related to the destruction of the tissues and body protein.

D : N ratio.

With phlorrhizin during fasting the average D : N ratio for each group was: 2.97 in the controls, 2.50 in those without the posterior lobe, 3.17 in the thyroidectomized and 3.50 in those with lesions of the tuber. In the hypophysectomized the ratio was very low (1.62) with the peculiarity that there was no rise on the third and fourth days as observed

in the other groups. On a fat diet the ratio rose in the hypophysectomized to 2.45 and to 3.55 in the controls. On a meat diet the ratio was similar in both groups, being 2.38 in the hypophysectomized and 2.80 in the controls.

Acidosis.

Dr Rietti has studied the acetonuria in pancreatic and in phlorrhizin diabetes in fasting and fed animals. These experiments, which are being published separately, show that this is always lower in the hypophysectomized dogs.

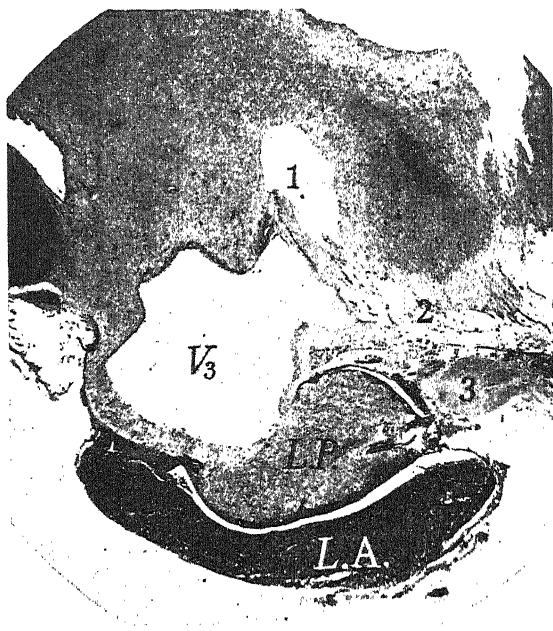


Fig. 1. Hypophyseotuberal region in a dog in which the tuber cinereum was cauterized. Sagittal section. *L.A.* anterior lobe of the hypophysis; *L.P.* posterior lobe of the hypophysis; *V₃*, third ventricle; 1, cystic cavity; 2, lacunar zone in the damaged region; 3, fibrous scar.

Histological examination.

Serial sections of the pituitary region were cut in all dogs which had had operations on the pituitary or tuber cinereum, the histological examination being undertaken with the technical assistance of Drs Lascano-Gonzalez and Sanmartino. In all the hypophysectomized animals the anterior lobe was missing, but there were always remains of the pars tuberalis adhering to the infundibulum, and in a few cases some

fragments of pars intermedia. In those dogs with lesions of the tuber there were necrotic remains or loss of substance, 4 or 5 mm. in length from the pituitary stalk to the posterior limits of the corpora mammillaria (destroyed), 2 to 4 mm. in depth and 3 to 4 mm. in breadth. In more than half the posterior lobe was destroyed; the anterior lobe was preserved complete or almost complete with its several types of cells.

DISCUSSION.

We have observed that hypophysectomized dogs readily develop hypoglycæmia during fasting or under the influence of certain substances, especially phlorrhizin. This hypoglycæmia must be due to the absence of the anterior lobe, because it is not observed in dogs deprived of the posterior lobe or thyroid or with lesions of the tuber. This hypoglycæmia and the fatal symptoms accompanying it do not occur if the dogs receive meat or sugar, but they are not prevented by fat.

The glycosuria set up by phlorrhizin in hypophysectomized fasting dogs is much less than in the other groups, which must be attributed to the anterior lobe deficiency. A fat diet does not modify it. The intake of meat increases the glycosuria although it is slightly less in the hypophysectomized than in the controls. It seems then that with phlorrhizin hypophysectomized dogs produce little glucose during starvation with no exogenous protein metabolism, while the production is larger when they are given protein in sufficient amount. This can be compared with the observation by Braier (1931) that hypophysectomized dogs have a lower endogenous protein metabolism, as shown by the following:

- (1) On a meat diet they eliminate less creatinine than controls.
- (2) During complete fasting or protein fast they eliminate 30-40 p.c. less nitrogen and 35 to 40 p.c. less creatinine than controls.
- (3) When *B. coli* vaccine is injected the protein catabolism increases less.
- (4) In diabetes due to total pancreatectomy their protein catabolism during fasting is less than if the pancreas is excised with the pituitary *in situ*; if they are fed they also live longer because of less wasting of their tissues.

It is reasonable to suppose that the fundamental change in hypophysectomized dogs is in their limited capacity to form glucose from the proteins of their tissues, in which case an important rôle of the anterior lobe would be that of regulator of endogenous protein metabolism.

It seems that when treated with phlorrhizin hypophysectomized animals are able to conserve or transform part of the sugar or its pre-

cursors formed from protein. This explains their very low D : N ratio when fasting and the low ratio when on a meat diet. In a previous paper we have shown that when after excision of the pancreas their blood sugar was not very high, hypophysectomized dogs could retain part of the sugar administered and their respiratory quotient rose.

The tuber lesion somewhat diminishes phlorrhizin glycosuria and nitrogen elimination during fasting. This can be attributed to a slight functional disturbance of the anterior pituitary lobe as shown by similar observation in the toad already quoted, especially if we remember that the tuber sends nerve fibres to this gland and that this in its turn seems to influence the tuber by a secretion which reaches it both through the stalk and by the blood stream.

Thyroidectomy does not affect phlorrhizin glycosuria in fasting dogs, reduces the nitrogenous elimination in fasting without phlorrhizin and reduces slightly that observed in fasting dogs under phlorrhizin. But the alterations caused by hypophysectomy are incomparably more pronounced than those following thyroidectomy.

SUMMARY.

1. In hypophysectomized fasting dogs phlorrhizin glycosuria is much less marked than in controls, in thyroidectomized dogs and in those without the posterior lobe of the pituitary. In the hypophysectomized there is an early fatal hypoglycæmia which does not occur in the other groups.

2. On a meat diet the glycosuria is much less in hypophysectomized dogs than in controls. Hypoglycæmia and death are prevented by feeding meat or sugar but are not prevented by fat.

3. The nitrogenous elimination in fasting is lower after hypophysectomy and after thyroidectomy than in the controls or after tuber lesions. The fasting elimination after phlorrhizin is less in the hypophysectomized than in the thyroidectomized, in those with tuber lesions, in the controls and in those without the posterior lobe. On a sugar or fat diet the fasting figures are only slightly modified, but on a meat diet the nitrogenous elimination of the hypophysectomized is nearer that of the controls.

4. In the hypophysectomized dogs the loss in weight is always more gradual. The D : N ratio and degree of ketosis are less.

5. It appears that the capacity to form sugar at the expense of the endogenous protein is greatly diminished in hypophysectomized dogs.

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KETOSIS IN THE PANCREATIC AND PHLORRHIZIN DIABETES OF HYPOPHYSECTOMIZED DOGS.

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IN the hypophysectomized toad total extirpation of the pancreas either does not produce diabetes or causes merely a slight rise in the blood sugar. The implantation of the anterior lobe of the pituitary definitely produces or increases the diabetes of the pancreatectomized toad, whether previously hypophysectomized or totally decerebrated [Houssay and Biasotti, 1931 *a*].

Work done by Houssay and Biasotti [1931 *b* and *c*] shows that the diabetes caused by pancreatectomy or by injection of phlorrhizin is considerably diminished in dogs which have previously been subjected to hypophysectomy. This diminution of diabetes is shown particularly as regards the glycosuria, blood sugar, time of survival and D : N ratio. The present data as to the excretion of ketones in the diabetic dog indicate also a decrease in the severity of the diabetes in hypophysectomized animals. They also show that the anterior lobe of the pituitary is concerned in the ability of the organism to form ketone bodies.

Animals and Method. 74 dogs were used. The samples of urine were taken almost entirely from the animals experimented on by Drs Houssay and Biasotti. The operative methods are described in the work quoted, from which the figures relating to the sugar in blood and urine given in Tables I to III have been taken.

The method of determination of the total ketone bodies was that of van Slyke [1917] modified by using only 10 c.c. of urine instead of 25 c.c., reducing in equal ratio the reagents used in the first part of the method.

RESULTS.

1. *Ketosis in pancreatectomized dogs.* Under this heading we include a summary of the data already published [1931] with the addition of the results obtained in six other dogs.

Examining Table I, it is seen that in the controls there is an average daily excretion of 76 mg. of ketone bodies per kg.; this is low compared

TABLE I. Ketosis in pancreatic diabetes in dogs.

	With hypo- physis excised (9 dogs)	Controls (6 dogs)	With tuber cauterized (5 dogs)
Daily average:			
Ketosis (acetone in mg./kg.)	21	76	31
Glycosuria (sugar in g./kg.)	1.05	3.00	2.04
Blood sugar (average mg./100 c.c.)	209	380	298

with the values obtained by Hedon [1930], who found on suspending the administration of insulin to a pancreatectomized dog the ketone bodies in 24 hours amounted to more than 0.5 g. per kg. per diem. MacLeod [1928] quotes data of Chaikoff, who found about 0.3 g. of ketone bodies per kg. excreted daily from the third to the sixth day after deprivation of insulin and food in pancreatectomized dogs.

Our dogs received insulin in the post-operative period during healing of the wounds. Each day they had 100 g. of fresh bovine pancreas and about 300 g. of meat. During the days when the observations recorded in Table I were made, they did not have insulin. All our determinations give lower ketone values than those obtained by the workers quoted, which must be attributed to the different treatment of the animals.

The average weight of the hypophysectomized dogs was 8.3 kg., the smallest 6.4 kg., the heaviest 11.9 kg.; that of the controls ranged from 6 to 15 kg., with average 9.3 kg.; the dogs with cauterized tuber were a little lighter (average 5.7 kg.). The pituitary had been removed several weeks or even months previously. The observations on the urine extended over several days, in three cases over three weeks or more.

TABLE II. Ketosis in phlorrhizin diabetes in fasting dogs.

	With hypo- physis excised (6 dogs)	Controls (6 dogs)	With tuber cauter- ized (7 dogs)	With thyroid excised (6 dogs)	With post- lobe removed (4 dogs)
Daily average:					
Ketosis (acetone in mg./kg.)	5	88	120	123	116
Glycosuria (sugar in g./kg.)	0.81	1.71	1.38	1.91	2.31
Blood sugar (average mg./100 c.c.)	70	89	92	97	80

Of the nine hypophysectomized dogs, which never received insulin, five excreted ketone bodies equivalent to 13 mg./kg. or less in 24 hours: two of the remaining four excreted 25 and 28 mg., which is less than the smallest amount obtained from any of the controls, and the other two 44 and 54 mg. In these latter four dogs the diabetes came on with great rapidity and intensity and there was a high blood sugar; the one that

gave 44 mg. had the highest degree of glycosuria of all (2.83 g./kg.). In three of the dogs with very low degree of ketosis the diabetes was slight with but little hyperglycæmia; from time to time in these animals hypoglycæmic attacks came on which were of some severity and finally caused death. The average ketone excretion for the whole group was only 28 p.c. of that for the controls, and the average blood sugar and average urinary sugar were, as shown in Table I, conspicuously lower than in the controls.

In the hypophysectomized dogs the total ketone elimination varied to some extent with the blood sugar and with the urinary elimination of sugar. Pancreatectomized dogs with lesion of the tuber cinereum (produced by galvano-cauterization from the pituitary stalk to the posterior limits of the corpora mammillaria) showed an average ketone elimination intermediate between that of hypophysectomized and of control animals.

A somewhat similar effect of lesion of the tuber cinereum has been observed by Houssay and Biasotti in the glycæmia of phlorrhizin diabetes, which seems to indicate a slight inhibition of the anterior pituitary. Three results are lower than the average for the hypophysectomized. The blood sugar is higher than in the hypophysectomized dogs.

2. *Ketosis in phlorrhizin diabetes during fasting.* Most of the dogs used were those experimented on by Drs Houssay and Biasotti, who showed that the diabetes due to phlorrhizin injection was diminished in hypophysectomized dogs. Before the experiments the dogs were kept in separate metabolism cages and received daily 400 to 500 g. of beef. At the beginning of the experiment they were starved, but were allowed water *ad lib.*; that day they received intravenously 1 g. of Kahlbaum's phlorrhizin dissolved in 25 c.c. of 1.2 p.c. sodium bicarbonate solution and on each succeeding day 1 g. of phlorrhizin suspended in 20 c.c. of olive oil was injected under the skin of abdomen or flank. Phlorrhizin was injected into controls and into dogs which had been hypophysectomized more than two weeks and up to as much as nine weeks before, or thyroidectomized eight weeks or more previously, or had lesions of the tuber cinereum which were three weeks or more old or had been deprived of the posterior lobe for two or three months. The observations on the urine of each day extended generally over four or five days, but sometimes over a period longer than that.

The results are shown in Table II, from which it can be seen that the hypophysectomized eliminate much less ketone bodies (5 mg./kg. in 24 hrs.) than the other animals (88-123 mg./kg.).

They also have a lower blood sugar and less glycosuria. There is

probably less production of sugar as the respiratory quotient and basal metabolism do not show an increase in the consumption of carbohydrate according to the experiments of Houssay and Biasotti.

3. *Ketosis in phlorrhizin diabetes in fed animals.* The total ketone bodies present in the urine of twenty-five dogs was determined, completing the work of Drs Houssay and Biasotti.

The food daily received by the dogs is shown in Table III. The phlorrhizin was given in the same way and amounts as described for the fasting dogs.

TABLE III. Ketosis in phlorrhizin diabetes in (a) hypophysectomized, (b) control dogs.

	Fed with					
	Meat, 300 g. daily		Sugar, 50 g. daily		Fat, 100 c.c. olive oil emulsion daily	
	(a) 5 dogs	(b) 4 dogs	(a) 5 dogs	(b) 4 dogs	(a) 4 dogs	(b) 3 dogs
Daily average:						
Ketosis (acetone in mg./kg.)	12	56	18	35	11	74
Glycosuria (sugar in g./kg.)	3.3	4.2	2.6	4.2	0.8	2.6
Blood sugar (average mg./100 c.c.)	92	95	89	104	56	114

On the whole, food increases the ketone elimination in hypophysectomized animals and decreases it in the controls (see Table III). In both groups the blood sugar and glycosuria are raised. The hypophysectomized always eliminate much less ketone bodies than the controls on a similar diet.

Meat diet. The hypophysectomized dogs show only slight increase in ketosis (from 5 mg. in fasting to 12 mg.) in spite of great increase in the glycosuria; the controls show slight diminution (88 mg. in fasting and 56 mg. on meat diet).

Sugar diet. The controls show much lower ketone elimination (35 mg.) than in fasting (88 mg.) in accordance with the known anti-ketogenic action of glucose. This action apparently fails in the hypophysectomized where the ketone elimination is more than three times that in fasting (18 mg., compared with 5 mg.).

Fat diet. The controls eliminate similar amounts (88 mg. in fasting and 74 mg. on fat diet). The hypophysectomized show a slight increase (from 5 to 11 mg.), but it is not possible to draw definite conclusions owing to the brevity of the observations as hypophysectomized dogs on a fat diet die rapidly in hypoglycæmia (on the second, third and fifth days), while they survive on meat or sugar.

GENERAL CONCLUSIONS.

1. Ketone elimination is notably less (1:3.6) in the pancreatic diabetes of hypophysectomized dogs than in the controls. It bears a relationship to the level of the blood sugar. It is intermediate in animals with lesions of the tuber cinereum.

2. The ketone elimination is much less (1:17) in the phlorrhizin diabetes of hypophysectomized fasting dogs than in the normal controls; in thyroidectomized animals, in those without the posterior pituitary lobe, and those with lesions of the tuber cinereum ketone elimination is greater than in the controls.

3. In hypophysectomized dogs on meat, sugar or fat diets the ketone elimination in phlorrhizin diabetes is always less than in the corresponding controls.

4. The intake of sugar diminishes the ketone elimination in the controls, but in the hypophysectomized there is a slight rise.

5. The ketone elimination of hypophysectomized fed dogs under phlorrhizin does not increase as much as does the glycosuria during meat intake.

6. Pituitary insufficiency always diminishes the urinary elimination of ketone bodies.

This work was suggested by Professor Houssay in connection with his researches on diabetes in hypophysectomized dogs. He gave me the opportunity of carrying it out and constant help and valuable suggestions for which I am deeply grateful.

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THE BLOOD CHOLESTEROL IN ANÆSTHESIA.

By A. C. GHOSE, *Research Scholar.**(Department of Surgery, Lucknow University.)*

MANY observations on the cholesterol content of the blood have been made in animals under various conditions. The effect of pregnancy [Chamberlain, 1929], of diet [Bloor, 1932], of injections of cholesterol [Chamberlain, 1928] has been studied and also the relation between it and the fat and lecithine in the blood [Bloor, 1921]. That anæsthesia also affects the blood cholesterol index has been appreciated; Ducheschi [see Gray, 1930] found an increase in the cholesterol in the serum of dogs after repeated administration of chloroform, and Gray [1930] determined the effect in rabbits of repeated administration of chloroform, urethane and paraldehyde. He found the amount of cholesterol in the blood much above normal after chloroform anæsthesia.

Certain experiments to be carried out on rabbits in which the use of chloroform, ether and urethane anæsthesia is necessary, made it desirable to determine the effect of these anæsthetics on the amount of cholesterol in the blood. Other tissues, such as the brain, spleen, liver, suprarenals, gall bladder, etc., are being similarly examined with a view to obtaining light on the physiological and pathological metabolism of cholesterol; but the results with these tissues are reserved for future publication.

METHODS.

Anæsthesia was produced in albino rabbits by inhalation of chloroform and ether by the open method. Anæsthesia was induced very slowly and maintained for 1 hour; and then the animals were killed by an overdose of the anæsthetic.

Urethane dissolved in water was given by injection into the peritoneum; the anæsthetic dose given was 0.8 g. per kg. body weight. After 1 hour another dose was given to kill the animal.

The cholesterol content of the whole blood was determined in the alcohol-ether extract as prepared by the method of Bloor [1916] using the Liebermann-Burchard reaction.

EXPERIMENTAL RESULTS.

Blood cholesterol in normal rabbits. The normal cholesterol content of the whole blood was found to range between 56 and 105 mg. per 100 c.c., the average value being 80 mg. The average amount of cholesterol in the serum was 48 mg. per 100 c.c. and ranged from 23 to 65 mg. The average values were calculated from the results obtained in 36 rabbits. The results of Grigaut and his co-workers, of Weidman and Suderman, Clarkson-Newburgh and Harnes, as quoted by Gray [1930], are in good agreement with those obtained by him and also by me.

TABLE I. Chloroform. Blood cholesterol in mg./100 c.c.

Rabbit No.	Before anæsthesia	During anæsthesia	After death
56	65	—	88
57	72	—	122
58	65	—	96
9	84	—	112
10	82	—	130
11	80	110	118
59	75	105	120
60	80	95	134
61	85	112	156
Average	76.4	105.5	119.5

The effect of chloroform anæsthesia (Table I). The blood cholesterol was determined in nine rabbits before anæsthesia and after death from chloroform. In four of them it was also determined during the anæsthesia. There was a slight but definite rise (average 23 mg.) in the blood cholesterol index during the anæsthesia, while after the fatal dose the increase was greater (average 43 mg. per 100 c.c.).

TABLE II. Ether. Blood cholesterol in mg./100 c.c.

Rabbit No.	Before anæsthesia	During anæsthesia	After death
1	76	—	115
2	62	—	130
3	65	—	125
4	78	95	105
5	85	100	120
6	82	85	94
7	80	90	114
Average	75.4	92.5	114.7

The effect of ether anæsthesia (Table II). Seven rabbits were used. The index was found to be raised after death from ether, significantly in all but one, but on the average from all seven animals by 39 mg., a result similar to that obtained after chloroform. Robinson [1929] found in

cats no change in the blood cholesterol after ether anæsthesia or, if any, a tendency for it to fall. But Mahler [1926] found that in man after ether anæsthesia of an hour's duration the amount of cholesterol in the whole blood always rose.

TABLE III. Urethane. Blood cholesterol in mg./100 c.c.

Rabbit No.	Before anæsthesia	During anæsthesia	After death
62	76	—	95
63	80	—	84
64	75	86	92
65	68	72	100
66	84	98	128
Average	76.6	83.3	99.8

The effect of urethane anæsthesia (Table III). This type of anæsthesia was used in five rabbits. In four of them there was no increase in the blood cholesterol, while in one, No. 66, it rose from 84 to 128 mg. per 100 c.c. During the anæsthesia the changes were not significant.

SUMMARY AND CONCLUSIONS.

An increase in the blood cholesterol index is found after a dose of chloroform or ether sufficient to cause death. During the course of anæsthesia the increase of the blood cholesterol is found to be no more than 20–25 mg. per 100 c.c. After death from urethane no increase in the blood cholesterol was found except in one case, which shows that there is a possibility of the blood cholesterol being increased.

In conclusion I thank Prof. R. N. Bhatia for the constant interest in the work.

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THE METABOLISM OF LACTOSE.

Part II. The blood sugar during lactation.

By L. B. WINTER.

(From the Department of Physiology, University of Manchester.)

FROM experiments described in the previous paper [Winter, 1931] the conclusion was drawn that, by relying only on estimations of reducing substances in the urine, the problem of the utilization of lactose by the tissues could not be satisfactorily settled. The present work was undertaken to discover whether lactose is a threshold substance in the blood. Best [1918] obtained from the blood of the cow a phenyl-osazone which he believed to be that of lactose, but the method by which the sugar was extracted was elaborate and not free from objection in that the blood was boiled with acid before and after removal of the proteins. The matter is discussed fully by Grevenstuk [1929].

Experimental.

The subjects from whom the blood was obtained were healthy nursing women, 3 to 5 days after parturition. Only those cases were chosen in which lactose was present in the urine: the amount was unimportant since, in the case of threshold substances, for any of the substance to be excreted in the urine, the amount in the blood must be above the threshold level. The blood was obtained by venous puncture, and defibrinated as collected: the blood sugar was extracted as described for rabbit blood [Winter, 1930]. Since it was undesirable that any considerable amount of blood should be drawn from a single individual, it was necessary to combine samples. Each batch of blood was therefore brought as quickly as possible to the stage of the dry lead precipitate, at which the sugar can be preserved indefinitely. When sufficient of the lead compound was available to give a satisfactory yield of sugar, the remaining stages were carried through.

The evidence obtainable from osazones derived from sugar mixtures was not likely to be of service; the objective therefore was the isolation in the crystalline condition of any lactose which might be present. When

glucose and lactose are in a syrup together with impurities, it is difficult to separate them; when crystalline it is easy to make use of the difference in their solubilities in methyl alcohol. After crystallization, the blood sugar was extracted with cold methyl alcohol; the residue should contain an increased proportion of lactose were this sugar present, and this would be indicated by the residue having a melting point above that of glucose and a specific rotatory power significantly below it.

The amount of sugar yielded by 271 c.c. blood was 99 mg., this by extraction with methyl alcohol was reduced to 47 mg. The melting point was 138–140° and the specimen showed $[\alpha]_{5461} = +110^\circ$ with a final value of + 60°. In another experiment 103 mg. sugar were obtained from 237 c.c. blood; after extraction 26 mg. remained with m.p. 138–140°, and initial and final rotations of + 115° and + 67° respectively. No evidence of the presence of lactose was obtained by fractional crystallization. The sugar syrup from 158 c.c. blood was drained on a porous tile when only partially crystalline; lactose being the less soluble sugar might be expected to crystallize before the glucose; 25 mg. of solid were obtained and this was further reduced by extraction with alcohol to 12 mg., m.p. 144–145°, $[\alpha]_{5461} = +108^\circ$, falling to + 56° after 18 hours.

An increase in the reducing power of the sugar mixture after boiling with dilute acid would not be proof of the presence of lactose, but identical values before and after hydrolysis would definitely exclude any disaccharide. Two such experiments were performed, the reducing power being estimated by the Wood-Ost method, and the results showed that no trace of lactose could be present. 107 c.c. blood yielded 40 mg. total sugar; the dry residue after extraction with methyl alcohol weighed 27 mg. and melted at 138–140°. 25 mg. were dissolved in 4.56 c.c. of *N*/5 HCl in a small tube, and 2 c.c. of the solution required 3.5 c.c. permanganate of such strength that the calculated glucose content was 10.5 mg., while the actual solid content was 10.9 mg. The tube was weighed, refluxed for 1½ hours, and re-weighed. A correction was made for the concentration due to the drop of water in the condenser. On titration 2 c.c. required 3.55 c.c. of permanganate (corrected).

In the second experiment 80 mg. of sugar were obtained from 155 c.c. blood. The residue after extraction (15.5 mg., m.p. 135–138°) was dissolved in 4.4 c.c. of acid. 2 c.c. required 1.95 c.c. of permanganate before and 1.85 c.c. (corrected) after hydrolysis. By working up the methyl alcohol solutions α glucose was obtained without difficulty. m.p. 145–146°. 17.6 mg. in 1.615 g. water ($c = 1.07$) showed $[\alpha]_{5461}^{13} = +120^\circ$ with a final value of + 61°.

Micro analyses (by Dr Ing. A. Schoeller, Berlin): C 39.87, 39.99; H 6.54, 6.59; $C_6H_{12}O_6$ requires C 40.00, H 6.66.

During the course of further work it was seen that the blood sugar of lactating women often yielded two distinct types of crystals. Although lactose had been shown to be absent, it was possible that traces of another sugar might be present, the most likely being galactose. The initial rotations of the residues after extraction with methyl alcohol did in fact support this hypothesis, for the values lay near to that for α glucose, although in previous work two recrystallizations were found to be necessary in order to obtain pure α glucose from rabbit blood. Galactose is less soluble than glucose, and would tend to be left in the residue after extraction of the dry mass with alcohol. The total sugar from 440 c.c. blood taken from four cases 4 days after parturition was extracted with cold methyl alcohol, and the residue was recrystallized from the same solvent. Two fractions were obtained.

Fraction 1: 32 mg. M.P. indefinite, 140–152°. The initial and final rotations were +138° and +77° respectively. C 39.91, 40.07, H 6.73, 6.75.

Fraction 2: 9 mg., melted gradually between 140° and 150°. $[\alpha]_{5461} = +146^\circ$, falling to +83° after 18 hours.

The high rotations, initial and final, and the raised though indefinite melting points in this experiment, might be accounted for by the presence of a small amount of impurity. The elementary analysis of fraction 1, however, was that required for $C_6H_{12}O_6$: a small quantity of galactose whose specific rotation and melting point are +170° and 168° respectively could account for the discrepancy.

1.13 g. of sugar was accumulated, taken from twenty-five cases at the same stage of lactation, 4 days after parturition. The sugar was extracted six times with methyl alcohol; there remained 4 mg. of residue which was discarded. The six extracts were worked up separately and yielded six crystalline fractions with the following physical properties:

Frac- tion	M.P.	$[\alpha]_{5461}$		C	H
		Initial	Final		
1	138–140	117	60	39.71, 39.91	6.75, 6.73
2	146–147	125	68	39.96, 40.27	6.87, 6.79
3	139–140	120	60	39.87, 40.15	6.70, 6.85
4	140–145	125	63	39.94, 39.75	6.72, 6.69
5	148–150	124	64	40.17, 40.20	6.77, 6.73
6	148–150	126	67	39.95, 40.06	6.79, 6.72

The figures do not show a progressive rise in the melting points and specific rotations of successive fractions as would have been the case had

any appreciable amount of galactose been present in the sugar. The insuperable difficulty when a composite specimen of sugar from many different sources has to be examined is that only in a few cases may the sugar have been present, and before this has been worked up it has been diluted with much material that is valueless. The question must be left undecided. In the case of the examination of the blood sugar for lactose, the negative result is at first sight remarkable, in that the sugar was present in the urine and must therefore have been in the circulation; but it would indicate that this sugar on absorption from the mammary gland is only present momentarily in the blood, and there can be no threshold for it.

SUMMARY.

Neither lactose nor galactose has been detected in the blood sugar during lactation; there is no threshold for the former sugar.

I am grateful to Prof. D. Dougal for permission to obtain material from his cases at St Mary's Hospital. The expense of this work was in part defrayed by the Government Grant Committee of the Royal Society.

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A NEGATIVE PHASE IN THE HEAT PRODUCTION OF MUSCLE.

BY W. HARTREE.

(From the Physiological Laboratory, Cambridge.)

IN a previous paper [Hartree, 1932, p. 284] it was stated that negative delayed heat production had been frequently observed after the contraction of a frog's muscle at a low temperature in the absence of oxygen. It was suggested that the result might be due to a technical error, most likely to a small increase in the weight of the muscle between the times of taking the "live" and the "control" curves. Such an increase of weight, which might be due to a shortening and thickening of the portion of muscle on the thermopile, was shown to produce an effect similar to that observed.

It was possible to test this explanation of the negative delayed heat by making "control" curves on the living muscle mixed with curves recording the response to stimulation. Dr E. Bozler (personal communication) has employed a high-frequency oscillating current for this purpose. Prof. D. T. Harris was kind enough to supply Prof. A. V. Hill with the valve oscillator shown diagrammatically in Fig. 1, which gives a current of such high frequency that the muscle does not respond to it and yet not so high that the "skin effect" is appreciable. By means of this the living muscle can be sufficiently heated for control curves to be taken without any sign of stimulation. It was tested and confirmed that heating a muscle in this way has no harmful effect on its subsequent response.

The heating current was applied for about 0.05 sec. by allowing a revolving arm to open a short circuit key, which promptly fell back as soon as the arm had gone by. While the key was open the high-frequency current passed through the muscle between the usual electrodes.

By taking sets of control curves interspersed with the live curves it is evidently quite immaterial whether the weight of a muscle alters progressively during an experiment or not. It is interesting, however, that in every one of several experiments the control curves for 0.05 sec., heating, taken later on during an experiment, fell from their maxima

faster than did those taken earlier, which can only be explained by the muscle losing weight during the course of an experiment. The gas in the chamber is saturated, so this loss of weight cannot be due to evaporation: presumably a certain amount of liquid gradually drains out of or away from the tissue. The loss of weight is probably quite small, but it can hardly be estimated. Its effect would be in the opposite direction to that required to explain away the negative delayed heat as a technical error. That explanation, therefore, is invalid.

The effect of this gradual change in the control curve is to make the negative delayed heat previously described rather too small. A live curve is analysed by the correct control curve taken at about the same time, and also by a more quickly falling control curve taken much later. Using the same procedure as in the paper referred to, the analysis of the initial heat is hardly affected by the difference between the two control curves, but the calculated values of the effect at subsequent times of the initial heat are too small in the latter case, and these, subtracted from the live curve, give a set of negative numbers which are numerically too small. These numbers, however, are then analysed by a 4 sec. heating curve (built up from the corresponding control curve) which also falls rather too fast, with the result that the calculated negative heat is only about $\frac{1}{2}$ p.c. of the initial heat too small. Although this is a considerable fraction of the total negative heat (about - 5 p.c. of the initial heat in the case examined) it is of no great account, being at about the limit of observation.

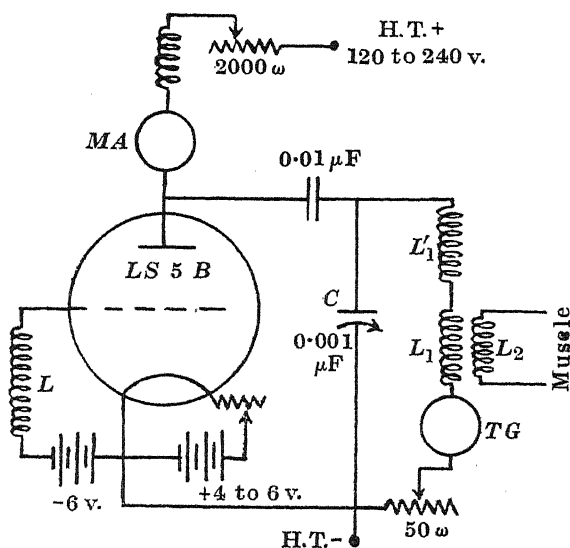


Fig. 1. Diagram of connections of high-frequency oscillator for heating without excitation. L and L_1 are magnetically coupled (variable), and L_1 and L_2 are magnetically coupled (variable). The milliammeter (MA) measures the H.T. current and the thermogalvanometer (TG) measures the oscillator generator current. The frequency is about 100,000 oscillations per second.

Using the new method of making curves of control heating, a number of further experiments were carried out, all on pairs of sartorii of English *Rana temp.*, at 0°C. , in nitrogen freed from oxygen by being passed over red-hot copper gauze. The low temperature is necessary since, at higher temperatures, the usual positive delayed heat previously described masks the negative.

The usual procedure was as follows. After about an hour (including dissection) in Ringer's fluid at room temperature the muscle, still in Ringer's fluid, was brought to 0°C. , oxygen being bubbled for about 30 min. and then nitrogen for another 30 min. The Ringer's fluid was then blown out with nitrogen, and nitrogen still passed slowly through the chamber while one or two preliminary stimuli were given. Although desirable it was unfortunately impossible to get a reliable result for the first stimulus, since the initial heat for this was usually abnormal, probably due to the muscle not being in the same position on the thermopile before the first stimulus as it was afterwards.

Two thermopiles were used to see if the negative heat was in any way a function of the thermopile. In the one used most frequently, made by Mr A. C. Downing and referred to below as thermopile *H* [see Hartree, 1932, p. 274], the muscle pulled on the metal frame on which the thermopile was mounted; there was thus a danger of some thermo-elastic effect in the frame as the "cold" junctions were held by it. [See Feng, 1932, p. 458.] Any effect of this, however, cannot account for more than a small portion of the negative heat since (1) it was found that a very much greater tension, applied to the frame at the same point as that at which the muscle pulled, than that exerted by the muscle, was required to produce galvanometer deflections comparable with those on which the negative heat depends; (2) the negative heat lasted for a much longer time than the thermo-elastic effect; and (3) when the tension had much diminished at the end of an experiment, the negative heat was always numerically greater, sometimes much greater, though the thermo-elastic effect must then have been less.

In the other thermopile (referred to below as thermopile *T*) there was no possibility of thermo-elastic effect, but it had two disadvantages: (1) it lost heat rather quickly, so the later readings were rather small; (2) the chamber was made of ebonite and it is strongly suspected that this, or defects in the tube leading to the chamber, may have supplied the muscle with a small amount of oxygen (possibly dissolved in the ebonite) because it was distinctly observable that the response when using this thermopile held up much longer than when using the former

one. This may well account for the result which is admitted that the second thermopile gave appreciably less negative heat than the first, as the numbers given subsequently will show.

RESULTS.

Care was taken in the calculation of the negative heat not to include any results if these contained early negative heat such as would occur if there were any inactivity in the outer layers of the muscle far from the thermopile [see Hartree, 1932, p. 284]. Such cases are easily detected, as the apparent negative heat then either cuts off the end of the relaxation heat or appears as a quickly falling negative rate only lasting for a second or two. If, on the other hand, there is a distinct break (*i.e.* no observable heat of any kind) for a second or two after relaxation, and if then the negative heat starts with an increasing rate, it is considered as genuine, even though this occurs before 4 sec. after a short stimulus. There was, however, in many cases a very good "wash-out" of the numbers in the analysis between the end of relaxation and 4 sec. (for stimuli not longer than 1 sec.). Even then, however, it is remarkable that the analysis of the later negative heat carried out in steps of 4 sec. and starting at time 4 sec. from the beginning of the stimulus, nearly always showed a maximum rate of negative heat between 4 and 8 sec. For this reason the initial analysis was carried on in a few cases for 8 sec. and a typical result is shown in Fig. 2, where, although there was no observable negative heat before 4 sec., its maximum rate was at about 6 sec.

The result is not greatly different for any time of stimulus from 0.1 sec. up to 1 sec. so long as the negative heat rate is expressed as a fraction of the initial heat; the amounts, however, are very different in different experiments, and in any one experiment the negative heat always increased for subsequent stimuli, being frequently much greater for the second record than for the first, the latter being usually taken after one or two preliminary stimuli of very short duration. This may perhaps account for the diminution of the positive delayed heat for successive stimuli observed at higher temperatures.

For longer stimuli, 4 to 5 sec., the total negative heat was always less than might have been expected (according to the usual increase just mentioned) and it ended earlier. This is no doubt due to the earlier occurrence of the positive delayed heat in such cases (as has been shown at higher temperatures [see Hartree, 1932, Fig. 2, p. 278]. The onset of the positive delayed heat masks the end of the negative heat. After a 10 sec. stimulus there was no observable negative heat, although for a

1 sec. stimulus taken before and for another after, there was large negative heat (-5 and -7 p.c.) going on for a long time (40 and 50 sec.). Such cases, therefore, were discarded, and the totals subsequently mentioned refer to stimuli of 1 sec. or less.

For an early stimulus, usually after one or two preliminary stimuli, the total negative heat was from 0 to -2 p.c., in all cases for thermopile

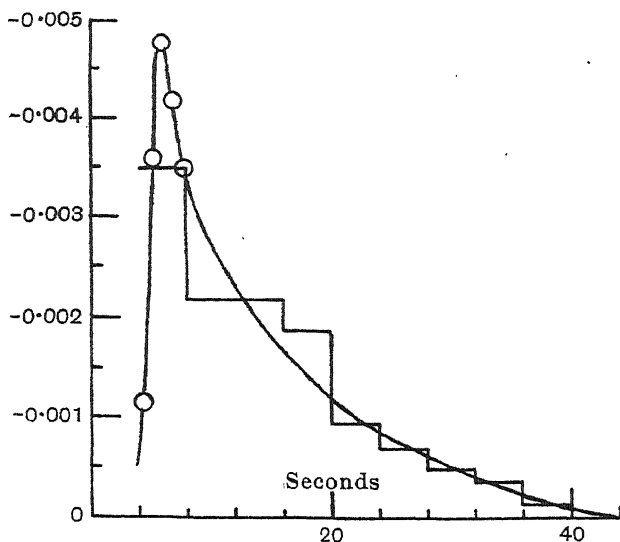


Fig. 2. Negative heat rate subsequent to a stimulus of 1 sec. at 0°C . (taken after several others). There was no observable heat of any kind between 2 and 4 sec. The stepped line shows the result of the usual analysis of the later heat by 4 sec. intervals. Circles show the result of the initial analysis being carried to 8 sec. by steps of 0.8 sec. The actual form of the curve, though smooth and similar to that in other cases, is hardly to be relied upon as it depends on very small differences. The end of the curve is not asymptotic with the base as a small positive heat rate supervened. The vertical scale is in terms of the initial heat per sec. and the total negative heat is in this case -5 p.c. of the initial heat.

T and in several cases also for thermopile *H*: for the latter, however, it was much more (up to -6 p.c.) in a few cases.

After several stimuli and a considerable time thermopile *T* gave a total of -2 p.c. in several cases, going up to -6 p.c. in a few cases (average $-3\frac{1}{2}$ p.c. for seven cases). Thermopile *H*, on the other hand, never gave numerically less than -4 p.c., and in four cases the total was up to -9 p.c. (average -7 p.c. for eleven cases). The averages are of no particular value beyond showing the different results arrived at by the

different thermopiles, since there is no indication that the maximum negative total was reached in any case—the positive delayed heat probably always interfered with it to some degree. If, as explained above, readings of thermopile *T* are open to some doubt, the maximum negative heat is probably about the same (8 p.c.) as that found for the positive delayed heat in the absence of oxygen for fresh muscle. [See Hartree, 1932, p. 278.]

As regards the distribution of the negative heat this appears to depend little on the length of the stimulus. After a long stimulus of 4 sec. there is quite a good “break” or “wash-out” between relaxation and 8 sec., so that the negative heat does not start with an exceptionally large early rate. The maximum negative rate in fact depends only on the total negative heat, being from -0.0007 to $-0.001 \times$ (initial heat per sec.) for each 1 p.c. of negative heat, and this occurs between 4 and 8 sec. after the end of the stimulus (including stimuli up to 4 sec.).

The time to completion of the negative heat is shown approximately by the following (rough average of about 35 observations):

Total negative heat: p.c. of initial	1	2	3	4	5	6	8
Time of completion: seconds	15	25	30	35	40	45	50

In a few cases the greater totals required 60 sec. for completion. Of course the time of the end of the slowly descending curve is very indefinite, and this would be considerably affected by traces of oxygen and by differences in the occurrence of the positive delayed heat after different stimuli, but the average of a large number of observations will give a good enough general idea.

It should be noted that the maximum tension for successive contractions fell from the start, and in the later records (after 2 or 3 hours) it was sometimes not more than half the original value; the initial heat also fell for the later records, though there was sometimes a distinct rise for the earlier ones, even though the maximum tension was falling. In every case the percentage fall of tension was greater, and in many cases very much greater, than that of the initial heat¹. It seems, therefore, that the state of the muscle, especially when the negative heat was large, was very different from that of fresh muscle: it must be understood, however, that the muscles were not irreversibly changed by their treatment, as in all but one of about 20 cases after several hours in nitrogen they were perfectly restored after being left an hour or two in oxygenated Ringer's fluid, the final response being often slightly larger than it was initially.

¹ As has been observed before [Hartree 1931, p. 13].

As for the cause of the negative heat observed little can be said. It is natural to associate it with the earlier stages of a known endothermic reaction, namely the restoration of phosphagen which takes place in muscles deprived of oxygen at the expense of energy liberated by lactic acid formation. It will be of interest to find whether at 0° C. the time relations of phosphagen resynthesis are at all similar to those of the negative delayed heat. If the positive free energy of lactic acid formation were greater than the negative free energy of phosphagen resynthesis, the former might in principle drive the latter, even though the total energy of the combined reaction were negative. In part at least of the cycle the endothermic process has at 0° C. outstripped the exothermic one.

SUMMARY.

1. By means of a high frequency oscillating current (about 100,000 cycles per sec.) a muscle may be warmed without being excited; this supplies a means, in myothermic experiments, by which "controls" can be made at intervals during a series of observations on living muscles.

2. This avoids the possibility of errors due to a progressive change in shape, weight or position of a muscle on a thermopile.

3. In a frog's muscle at 0° C. after a short tetanus, there is a negative delayed heat production which is usually very small for early stimuli and this increases, for later stimuli, up to about 8 p.c. of the initial heat. This heat is complete in 30 to 60 sec. when it is followed by the usual positive delayed heat which, at higher temperatures, comes on so quickly as to mask it completely.

4. This negative delayed heat may be associated with the endothermic restoration of phosphagen, but further experiments on the time relations of that process are necessary before the connection is certain.

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THE HEAT PRODUCTION OF CRUSTACEAN NERVE.

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HILL in 1929 investigated the heat production of crab's nerve. The total heat per sec. of maximal stimulation was estimated to be at least 2.5×10^{-3} cal./g. of moist nerve, and a clear division of this heat into an initial and a recovery phase was brought out by analysis, the former representing about $2\frac{1}{4}$ p.c. of the total heat. In his work, however, photographic recording was not used, and analysis was only carried out in 5 or 6 sec. units. It was expected that finer details of the course of the heat production would be revealed by the use of photographic recording and by analysis in shorter units.

A number of points, moreover, in the energetics of crab's nerve remained to be examined: (1) the "optimal" stimulus; (2) the effect of frequency of stimulus; (3) the heat per single isolated impulse; (4) whether the course of the heat production in nitrogen is different from that in oxygen; (5) whether the natural state of steady activity revealed in the steady contraction of its attached muscle can be artificially realized by a sufficiently slow rate of stimulation; and (6) its resting heat production. The methods and technique developed recently by Prof. A. V. Hill [1932] for an investigation of the heat production of frog's nerve supplied the means for carrying out the programme described.

PROCEDURE.

Three thermopiles were employed: for the determination of resting heat an old silver-plated differential thermopile [see Beresina, 1932] and a new mica-faced silver-plated one: for heat production due to stimulation the new soldered thermopile referred to by Hill [1932, p. 112] as not ready in time for his experiments. A Kipp Zc galvanometer (period 5.5 sec.) was used in most experiments both for resting and stimulation heat production, occasionally a Zd (period 3.8 sec.) for the latter to gain quickness. Resting heat production was studied in a gas-heated water thermostat at 20.7° C., while a Dewar flask was employed for stimulation experiments. Stimulation was always by repetitive condenser charge and discharge with the aid of a commutator [Hill, 1932].

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The limb nerves of *Maia* were employed throughout. Six to eight nerves were required, and on a warm day nerves dissected first were kept in a sea-water bath at about 12° C. Usually two persons did the dissection together, and mounting of the nerves on the thermopile was begun by one person as the last nerve was being dissected by the other. After some practice the total time from the beginning of dissection to the placing of the thermopile with mounted nerves in the Dewar flask was about 30 to 50 min. Considering the rather rapid deterioration of crab's nerve, especially at higher temperatures, it is important that dissection should be quickly done. The nerves were then soaked in oxygenated sea water in the thermopile chamber for about 20 min., which suffices for equalization of temperature, and apparently also for removing the depressed excitability due to dissection and handling. The sea water was then sucked out and the chamber filled with moist oxygen. Usually in another quarter of an hour the conditions would be sufficiently steady to begin observations.

Photographic records were always taken in experiments in which analysis of heat production was intended.

OPTIMAL STIMULUS.

For the theory of the optimal stimulus and its importance in connection with the question of "heat leak" in neurothermic investigations we refer to Hill [1932]. In the study of crab's nerve heat the use of stimuli with minimal energy is necessitated by the facts (i) that crustacean nerve is easily injured by strong stimuli, and (ii) that, if stimuli of improper characteristics are used (*e.g.* break induction shocks), the energy in them may need to be too great.

In Fig. 1 are three curves from three separate experiments, relating the size of response to the electrical capacity of stimulus at constant energy. The curves do not possess such perfect symmetry as those for frog's nerve given by Hill [1932], but the existence of an optimal capacity is shown beyond doubt. In *A* and *B* the resistance of the stimulating circuit was 1560 ohms, in *C* 840 ohms. Reading off the capacity (*F*) corresponding to maxima from the curves, the optimal values of *RF* in the three experiments are 1150, 1290 and 940 respectively, averaging (say) 1200. In subsequent experiments the stimulus was always made to possess an *RF* in the neighbourhood of this value. It may be noted that the optimal *RF* for crab's nerve is about six times that for frog's nerve [Hill, 1932].

In cases, such as this and the next, in which no analysis was necessary, the size of response was determined as follows. The ordinates at the fourth, fifth and sixth seconds of the photographic record of each response were measured and their sum taken. This sum was used as the measure of the response. It was assumed that the curves of heat production due to stimuli of a given duration are all of the same shape, differing only in size, so that the corresponding points of all curves are comparable. The reason for choosing the fourth, fifth

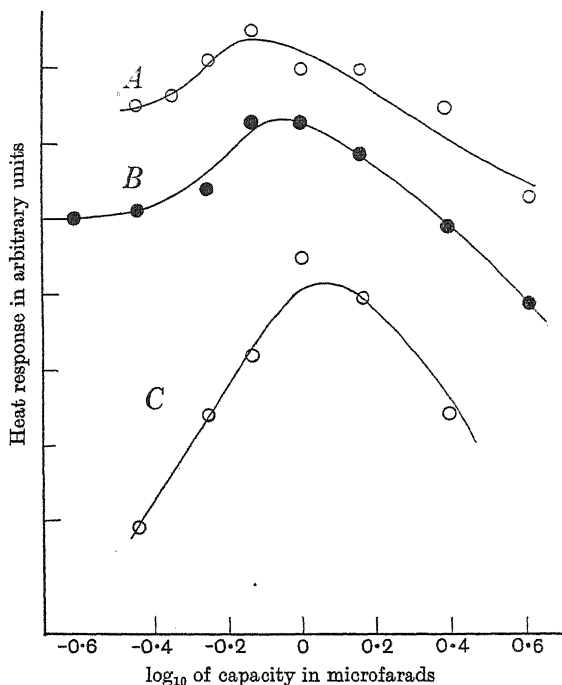


Fig. 1. Three determinations of optimal stimulus at about 17° C. Curves A and B were obtained with 1 sec. stimuli at about 90 per sec., C with 2 sec. stimuli at the same frequency. In each determination the energy of the stimulus (proportional to FV^2) was kept constant. The resistance (R) in series with the stimulating condenser (F) was 1560 ohms for A and B and 840 ohms for C. The product of the capacity in microfarads corresponding to the maximum of the curves and the resistance in series with it, or in short the optimal RF , was 1150, 1290 and 940 for A, B and C respectively, averaging about 1200.

and sixth seconds readings is merely that they are the earliest points on or near the horizontal limb of the curve (see Fig. 3, A) where measurement is easier for two reasons: (1) the portion of curve concerned is nearly flat so that a small displacement in time will introduce no error; (2) its proximity to the beginning of the curve reduces error due to any inaccuracy of the base line. The assumption stated may be inexact for long stimuli since varying degrees of failure during the stimulus would occur for different frequencies; for 1 or 2 sec. stimuli, however, it must be very nearly true.

FREQUENCY OF STIMULATION.

There are serious difficulties in determining the effect of frequency of stimulation upon the heat production in crab's nerve. Its rapid spontaneous deterioration and its ready fatigability tend to make successive observations not strictly comparable. Even in a stimulus as short as 4 sec. crab's nerve often shows considerable decrease in the rate of heat production during the stimulus (see below, section on analysis). Obviously long stimuli must be avoided. Fortunately with good nerves it was possible to obtain a good-sized response from stimuli as short as 1 or 2 sec. The use of such short stimuli reduces the effect of fatigue to a minimum, which was further compensated by taking observations first in increasing, then in decreasing frequencies, and using for each frequency the average of the two observations.

Nine experiments were made at various temperatures, but to avoid overcrowding only the results of five, all reduced to a maximum of 300, are plotted in Fig. 2, *A*. The points include three experiments made at about 16.9° C., and the frequency scale is for this temperature. The two other experiments included were made at 10° C. and 11.3° C., and their real frequency scales are $\frac{1}{2}$ and 1/1.8 respectively of that used in the plot. The fact that results obtained at 16.9° C. and 10° C. can be plotted together by a mere adjustment of the frequency scale shows that temperature, in crab's nerves as in frog's nerves [Hill, 1932] and muscles [Feng, 1931], only alters the time scale. The Q_{10} calculated from this relation is 2.9.

From the optimal stimulus data the excitation time of crab's nerve appears to be about six times as long as that of frog's nerve. This would lead one to expect that the maximum frequency of stimulation also for crab's nerve would be very much lower than that for frog's nerve. Comparing, however, Fig. 2, *A* of the present paper for crab's nerve at about 16.9° C. with Fig. 5 of Hill's [1932] for frog's nerve at 21.3° C. it will be noted that the difference in the frequency scale falls very short of expectation. The maximum frequency for crab's nerve at 16.9° C. may be taken to be about 200 per sec.; at 21.3° C., on the basis of a $Q_{10} = 3$, it would be about 320 per sec. This does not differ much from the maximum frequency of about 400 per sec. for frog's nerve at 21.3° C. (The value 400 per sec. is used for comparison, since the increase of heat response caused by frequencies higher than this is very slight. See Hill, 1932, Fig. 5.)

The large difference in optimal stimulus and the small discrepancy

in maximum frequency between frog's and crab's nerve need not, however, be contradictory. The optimal stimulus depends primarily upon the "excitation time"—indeed it can be derived from the strength-duration curve of nerve excitation, except for the complication that the optimal stimulus of the present work is determined for tetanic stimuli of 1 to 2 sec. duration, while the strength-duration curves are generally found by

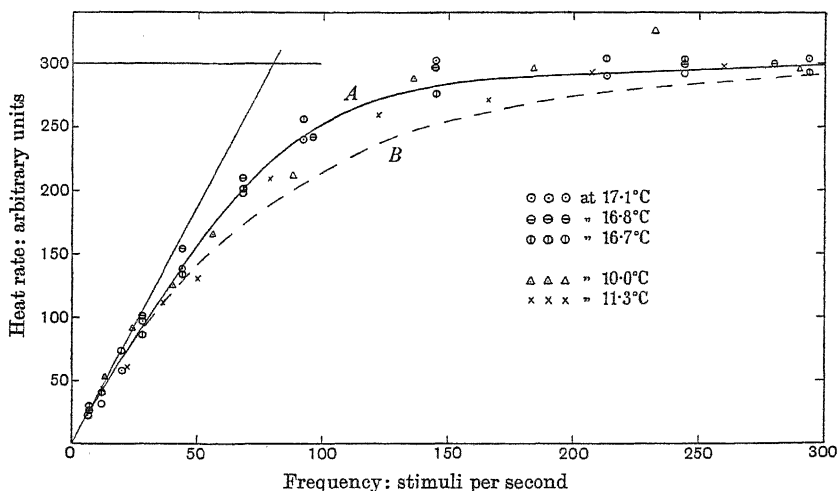


Fig. 2. The relation between heat production of nerve and frequency of stimulation. *A* is the mean curve for crab's nerve drawn through the points of (a) three experiments made at about 16.9° C., and (b) two at 10° C. and 11.3° C., the frequency scales for the latter having been multiplied by 2 and 1.8 respectively in order to plot them together with the three experiments at the higher temperature. The maxima of the curves of individual experiments have all been arbitrarily reduced to 300. Observations made at widely different temperatures are seen to lie sensibly on a single curve after an adjustment of time scale. *B* is Hill's [1932, Fig. 5] mean frequency curve for frog's nerve reproduced after reduction to the same maximum of 300 with its time scale for 21.3° C. multiplied by 0.72 so as to make the reading for frequency 25 per sec. lie on curve *A*. The divergence between *A* and *B* is definite, showing that the results from frog's and crab's nerves cannot be made to coincide, as those from the same nerve at different temperatures can. The tangent to curve *A* at the origin intersects the level of maximum heat production at a point whose horizontal coordinate is 80 per sec.

single minimal shocks. The frequency effect would be primarily related to the refractory period, or the curve for recovery of excitability after the transmission of an impulse. While on general grounds in similar structures quick excitation time is expected to be associated with short refractory period, they may not run parallel in structures so different as medullated and unmedullated nerve.

Another comparison is possible between the frequency curves of frog's and crab's nerves. If the nerves of frog and crab differed only in quickness, a proper adjustment of their frequency scales should bring their results to lie on a single curve, as is the case with the same type of nerve at different temperatures. Curve *B* in Fig. 2 is the mean frequency curve given by Hill [1932, Fig. 5] reduced to a maximum = 300, and with its frequency scale for 21.3° C. multiplied by 0.72 so as to make the reading corresponding to a frequency of 25 per sec. lie on curve *A* of Fig. 2. The divergence between curves *A* and *B* is obvious, and no adjustment of the frequency scale could abolish it. The basis for this is probably that frog's sciatic nerve has a more heterogeneous structure, consisting of fibres of a much wider range of diameter than crab's leg nerve. An appreciable fraction, in frog's nerve, of fibres of greater diameter (capable therefore of responding more frequently) would cause the curve to continue to rise after the response of the rest of the fibres had reached its maximum. If crab's nerve were more or less homogeneous, no such prolonged rise would occur.

THE ANALYSIS.

(1) *Short stimuli in oxygen.*

The analysis of short stimuli was always made in 1 sec. time units by means of 1 sec. heating controls. For the latter the mean of three or four curves was generally used. The number of live curves used for analysis varied for different durations of stimulus and in different experiments. For 1 sec. stimulus the sum of three to six curves was generally employed; for 4 sec. or longer stimuli, one to three curves. Compared with the case of frog's nerve such numbers seem small. They should, however, give accuracy enough, considering the size and smoothness of the records obtained (see Fig. 3), and the great susceptibility to fatigue and the rapid spontaneous deterioration of crab's nerve render it impossible to make a large number of records, as is feasible with frog's nerve. Generally, stimuli of durations from 1 to 4 sec. were given at intervals of 8 to 12 min., and four or five similar records of 4 sec. stimulus were about the most that could be secured.

Fig. 3 shows typical records of heat production due to short stimuli: *A*, *B*, *C* are 1 sec., 4 sec. and 8 sec. stimuli respectively, while *D* is the 1 sec. heating control by means of which the stimulus curves are to be analysed. They are all from one experiment made at 15.5° C. Fig. 3, *E* is another 4 sec. stimulus from an experiment at 20.5° C.

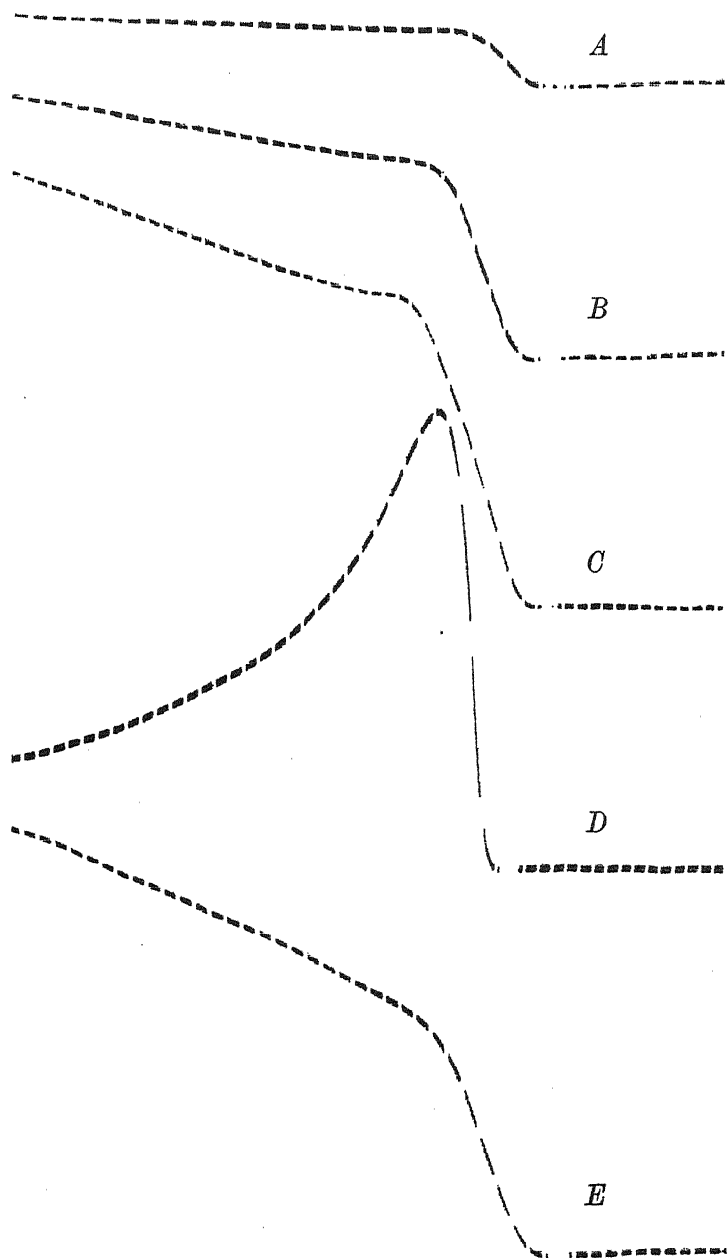


Fig. 3. Photographic records of heat production. *A*, *B* and *C* are 1, 2 and 4 sec. stimuli, 92 per sec. at 15.5°C . *D* is a 1 sec. heating control due to $380 \times 10^{-6}\text{ cal./g}$. This is intended also to convey some idea as to the sensitivity of our galvanometer-thermopile system. *E* is another 4 sec. stimulus at 20.5°C . Compare *C* and *E*. The higher rate of recovery heat production at the higher temperature is evident from the records without analysis. Time marks every second.

In Fig. 4 are shown two analyses of 1 sec. stimulus, and two of 4 sec. at different temperatures. Not only is there no ambiguity concerning the presence of initial heat, such as might exist in the case of frog's nerve at high temperatures, but the two phases of heat production, the initial

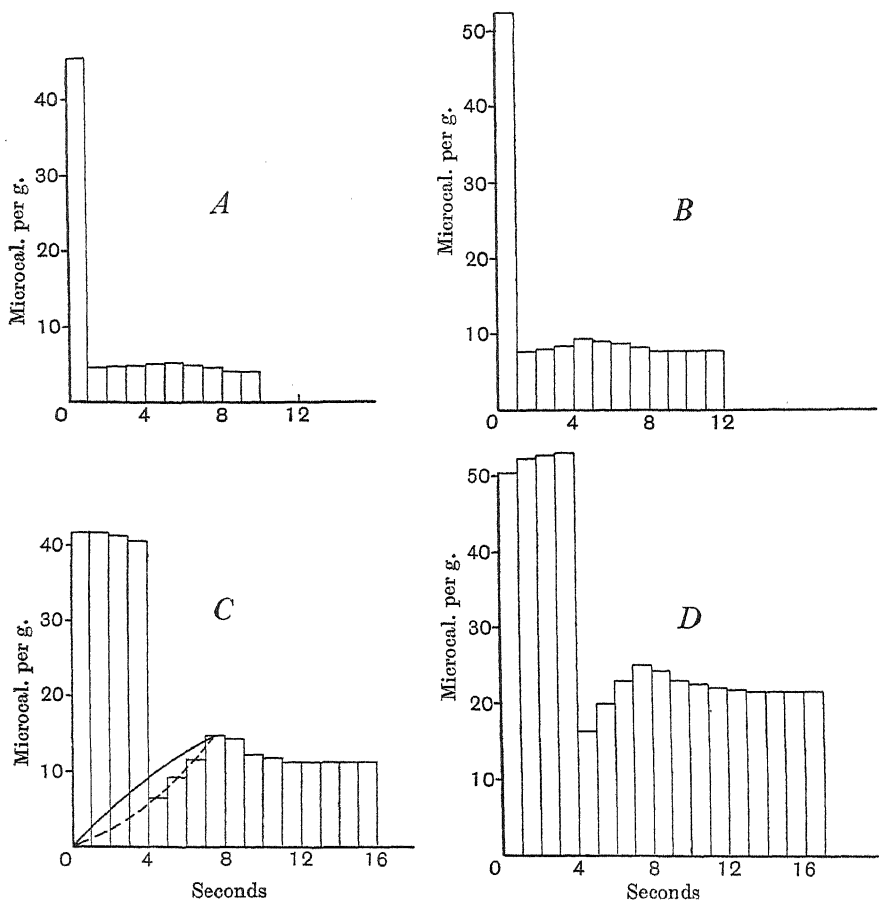


Fig. 4. Analyses of heat production of crab's nerve due to short stimuli in oxygen: *A* and *C* are 1 and 4 sec. stimuli, 92 per sec. at 15.5° C., *B* and *D*, the same at 20.5° C. See text for further description and comments.

and the recovery, are here almost distinct from one another. This is partly due to the relatively very large initial heat and partly to the fact that the maximum rate of recovery is not reached until some seconds after the stimulus is over, so that at the end of the stimulus there is a very abrupt and striking fall of heat rate followed by a "hump"

representing the recovery maximum. After the "hump" the recovery heat rate continues at a practically constant level for a period of at least 30 to 40 sec. That the rate of recovery heat production after a stimulus is higher at higher temperatures is evident from comparing analyses *A* and *B*, or *C* and *D* of Fig. 4. But to show this, analysis is hardly necessary: a mere inspection of photographic records is sufficient; compare for instance *B* and *E* of Fig. 3.

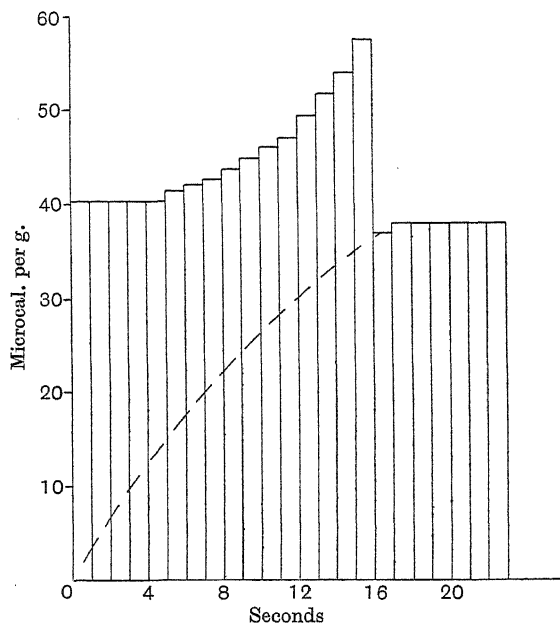


Fig. 5. Analysis of a 16 sec. stimulus, 92 per sec. at 18° C. Its somewhat unusual appearance during stimulation is due to the fact that the initial heat rate is decreasing, at first rapidly, then slowly, while the recovery heat rate is steadily increasing. The blocks above the dotted line may be taken as approximately representing the initial heats, and those below it the recovery heats in the successive seconds of stimulation.

In frog's nerve, at temperatures in the neighbourhood of 20° C. and using stimuli of about 200 per sec., there is always a great increase of heat rate during the course of a stimulus, indeed even for stimuli as long as 4 min. or more. This is, of course, largely due to the quickly increasing recovery heat summed together with the initial heat, but also to the relative constancy of the latter, as an inspection of Hill's analyses [1932] will show. In the case of crab's nerves, even in a stimulus as short as 4 sec., when the frequency of stimulus is over 60 per sec., there is either

only a slight rise in the heat rate or, as is more commonly the case, an actual fall. From the frequency curve it can be seen that a frequency of 60 to 90 per sec. is still a long way from the maximum for crab's nerve, and is relatively not higher but lower than 200 per sec. for frog's nerve at similar temperatures. Thus crab's nerve stimulated even at moderate frequencies may show pronounced "fatigue" in as short a time as 4 sec.

Fig. 5 shows the analysis of a 16 sec. stimulus at 92 per sec. at 18° C. The shape of the analysis appears unusual but easily intelligible once it

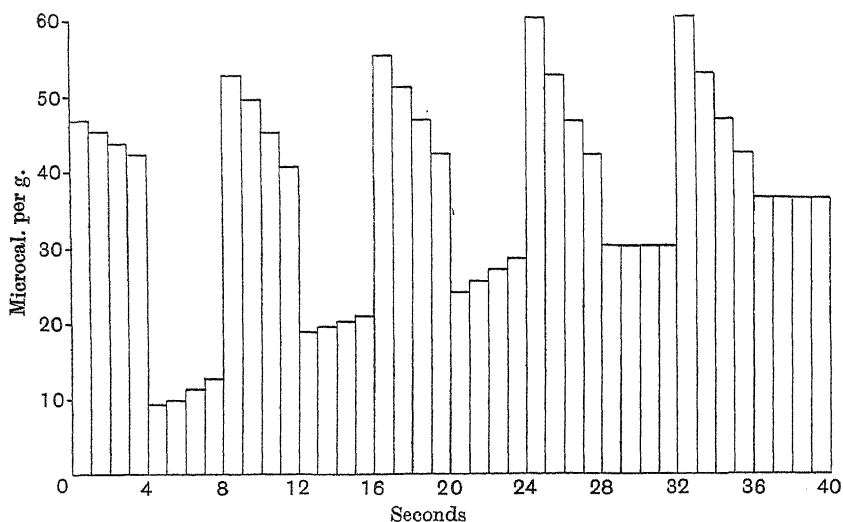


Fig. 6. Analysis of heat from intermittent stimulus, 4 sec. on, 4 sec. off, taken with Zd galvanometer. Frequency 92 per sec., at 17° C. Note the increasing degree of failure in successive periods of stimulation and the conspicuous though brief recovery after each rest interval.

is remembered that during the course of the stimulus the recovery heat rate is continuously increasing while the initial heat rate is decreasing, rapidly at first, then slowly. Clearly the sum of the recovery heat and the initial heat changing respectively in the manner described will result in the peculiar course of heat production as shown. In Fig. 5 the blocks below the dotted line may be taken as approximately representing the recovery heat rates in successive seconds of the stimulus, those above it, the corresponding initial heat rates.

Concerning the progressive failure of the initial heat rate, the following type of experiment is of interest. Instead of giving a 16 sec. or longer stimulus continuously, it may be split into 4 sec. periods, separated by

4 sec. gaps. The result of such an experiment is given in Fig. 6. We see (a) the first 1 sec. block of each 4 sec. stimulus is always higher than the last block of the preceding 4 sec. stimulus, showing that a rest of even 4 sec. can bring about considerable recovery; (b) while in all five periods of stimulation there is distinct failure of initial heat rate, the failure is more rapid in the later periods, showing the accumulated effect of previous stimulation, the recovery achieved in 4 sec. rest being very incomplete.

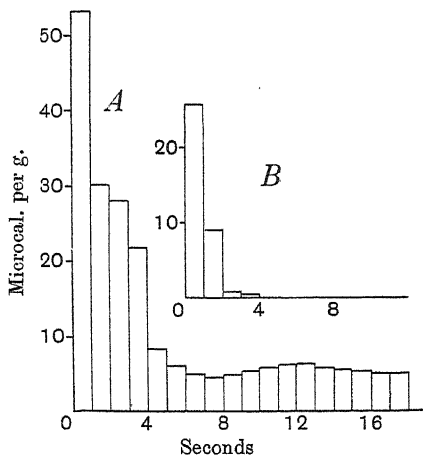


Fig. 7. Showing the very pronounced failure of crab's nerves at lower temperatures when stimulated at relatively high frequency. *A*, 4 sec. stimulus, 92 per sec. at 10.5° C. Note the striking decline of initial heat during the stimulus and also that the heat rate at the end of the stimulus did not fall suddenly to a low level rising again immediately afterwards, but dropped to a level which further decreased for 4 sec. before showing the usual rise. Compare this latter feature with Fig. 9 and see text for its discussion. *B*, 4 sec. stimulus, 60 per sec. at 0° C. The failure is striking. The last 2 sec. stimulation was hardly effective at all, and after the stimulus there was no significant recovery heat production up to the end of analysis. Scale same as *A*.

At lower temperatures the failure of crab's nerve during stimulation is very much more pronounced. In Fig. 7 are *A*, the analysis of a 4 sec. stimulus at 92 per sec. at about 10.5° C., and *B*, that of a 4 sec. stimulus at 60 per sec. at 0° C. In *A* the initial heat drops sharply after the first second and continues to fall in the course of the 4 sec. stimulation. The recovery heat at this temperature, as is to be expected, is produced at a relatively lower rate, reaching a maximum also somewhat later. The later maximum, however, is also due to another cause in this particular case, as will be explained later on. At 0° C. the failure of crab's nerve is striking. The record of which the analysis is shown in Fig. 7 *B* is very

small, but smooth, and as far as we could make out, Fig. 7, *B* is the approximate solution. After 2 sec. the heat production has become practically nothing and continues to be so until at least the twelfth second. The recovery rate is so slow that the nerve seems not in a position to respond to many stimuli in succession. That no irreversible damage was caused by the cold was found by warming the nerve up again to 17° C. when it gave good normal responses. Hill [1932] found that frog's nerves, too, will not work well if suddenly transferred to 0° C. Whether the failure at 0° C. in crab's and frog's nerve are of the same nature it is difficult to say. A number of *Maia* were placed in an ice box in the hope of producing acclimatization as in the case of frogs, but they all died in the box after one day. Perhaps the sudden transference from their normal environment of about 13° C. to 0° C. was too drastic a procedure. Facilities, however, for keeping them at intermediate temperatures and gradually working down to 0° C. in steps were not at our disposal. One possible contributory cause of the poor survival of the crabs in the ice box might be that the basin of sea water in which the crabs were kept was not aerated.

The analyses in Fig. 4 we present as the most typical results. In Fig. 7, *A*, coming before the "hump" representing the maximum of recovery heat rate, there is a small tail piece at the end of the stimulus which cannot possibly be fitted into the course of recovery heat production if the latter is to preserve any simple form. Such a tail piece occurred in about 30 p.c. of our records and appeared to be usually, but not always, related to a very pronounced failure of heat production during the course of the stimulus when the latter was longer than 1 sec. On the other hand it occurred relatively most frequently in analyses of 1 sec. stimuli, three out of seven cases, while in analyses of 4 sec. stimuli it occurred only in four out of sixteen. Further, in analyses in which it occurred the "hump" came later. Fig. 8 shows the analysis of a 2 sec. stimulus with conspicuous tail piece and delayed recovery maximum.

It is well known that an isolated crab's leg is capable of tonic contraction, and Barnes [1930] has reported persistent discharges in the motor nerve of *Cancer pagurus* as a result of cutting, pinching, ligaturing or traction. It is conceivable then that if the stimulus used in some of our experiments had been too strong, some injury might be produced at the site of stimulation, so setting up a short period of persistent discharges after the stimulus is over. This, if true, would account for the tail piece under consideration. Unfortunately the suggestion did not stand experimental test. In one experiment, after a number of records with the usual stimulus, the strength of stimulus was increased fifteen times. The result turned out to be diametrically the opposite of that expected: the analysis of some of the records taken with the usual stimulus showed the tail piece, while that of records obtained with the unusually strong stimulus did not. Persistent discharges aside, there appears no other way of understanding the tail piece as a physiological phenomenon. To compare it with the delayed anaerobic heat production in muscle seems hardly justifiable.

It remained therefore to be seen whether the tail piece could be regarded as an error. In muscle it has been confirmed [Blaschko, 1930] that if a piece of filter paper be interposed between the thermopile surface and the muscle there will appear an artificial

delayed heat production. Now if, of the six to eight nerves used, the centrally placed ones, viz. those directly lying on the hot junctions, were inactive during a short stimulus, the effect would be very similar to having a piece of filter paper in between the thermopile and the nerves, and delayed heat production or tail piece would be obtained.

That such is actually the case was shown by the following experiment. Six nerves were carefully dissected and three mounted on each side of the thermopile surface. Four records of 2 sec. stimulus and three control curves having been obtained, the middle nerve on each side was partly crushed below the stimulating electrodes. Records of 2 sec. stimuli and controls were again taken with everything precisely the same as before. Fig. 9 gives the analyses of the records before and after damaging the central nerves. The result is clear-cut:

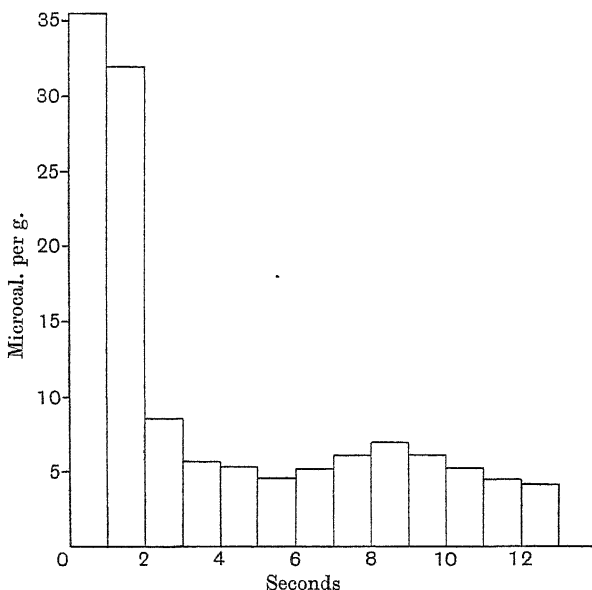


Fig. 8. Analysis of heat from 2 sec. stimulus, 90 per sec. at 15.7° C. showing the extra falling bit of heat production following the end of stimulus (or tail piece) and delayed recovery heat maximum.

analyses such as that of Fig. 8 can be produced artificially. The reproduction goes down even to details: there is the pronounced failure before the tail piece and the hump representing the recovery maximum is also delayed.

In the case of frog's nerves the chance of a large part or the whole of a nerve being inactive, assuming nerves from healthy animals to begin with, is small, frog's nerves being so resistant. With crab's nerves, however, the chance is larger. In the first place some fibres must have been cut or torn if a nerve was cleanly dissected, or else some tissue tags must have remained attached to it when its isolation was not so cleanly done; in either case a certain amount of inactive matter would be present in the preparation. In the second place, of the nerves placed on the thermopile some may have deteriorated more rapidly than others, and at the time of stimulating they may have been only partially active: such nerves may happen to have been centrally placed. Thirdly, since an over-maximal stimulus

is not safe [Furusawa, 1929], the approximately maximal stimulus employed may not have been adequate for some of the nerves, and these again may have been centrally placed. The experiment with the unusually strong stimulus related above, while failing to achieve its original purpose, is of interest in this connection. In that experiment the usual stimulus was most probably too weak for a good many fibres, particularly for those of the nerves occupying a central position, so producing a response whose analysis showed the tail piece, while the unusual stimulus, strong enough to stimulate all the nerves, also got rid of this appendage.

It may be objected to this explanation of the effect discussed that the converse case of apparent negative heat appearing at the end of the stimulus should also sometimes occur:

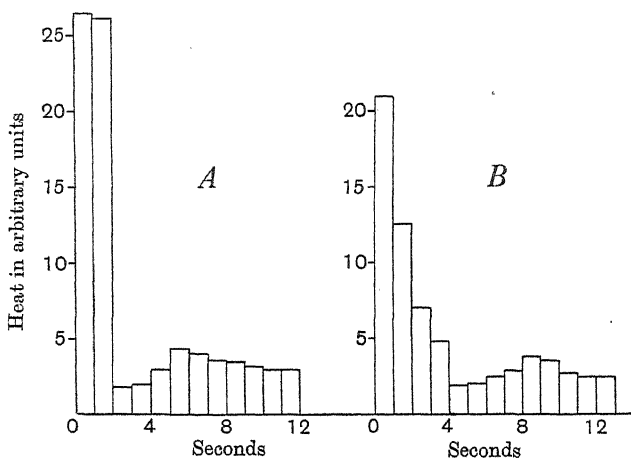


Fig. 9. Experiment for the elucidation of the tail piece shown in Fig. 9. *A*, analysis of 2 sec. stimulus taken when all nerves on the thermopile were presumably excitable and active; it is simple in appearance without the tail piece. *B*, analysis of 2 sec. stimulus precisely the same as *A* but obtained after the central nerve on each surface of the thermopile had been damaged; the artificial delayed heat production (or tail piece) is most obvious, and the maximum of recovery heat is also delayed. There is besides the very pronounced failure during the stimulus. Thus, by merely damaging the nerves occupying the centre position on each thermopile surface, it is possible to reproduce analyses like that in Fig. 9.

the central nerve, the one on the thermo-junctions, might be more and not less active than the average of the rest. As a matter of fact such cases do appear but not very obviously or often. Fig. 4, *C* may serve as an instance. There the course of the recovery heat production found by analysis follows the dotted line: the true course is most probably more like the full line; negative heat would account for the discrepancy.

The reason for the relative infrequency and the smallness, when it appears, of the converse effect is probably as follows. In determining the form of the record, the two nerves lying actually on the warm junctions of the thermopile exercise a more immediate influence than the rest. Their activity, like that of any other single nerve, may vary from zero on the one side up to a certain maximum on the other. Since, however, an experiment is always discontinued when the nerves are showing obvious signs of failure, the average of the six or eight nerves used must always be rather high. Representing maximum

excitability by 100, that of the pair of nerves on the junctions may vary from 0 to 100, while the average of all the nerves would never be much less than 70. It is just as likely that the central nerves on the junctions should be above or below the rest in respect of their excitability, but the amount by which they can be above is much less than the amount by which they can be below, and it is only those cases in which the difference between the central nerves and the rest is large enough which will show any obvious sign of the effect under discussion. It is natural, therefore, that the converse case of apparent negative heat following the stimulus should be relatively infrequent and the amount, when it occurs, be small.

(2) *The maximum recovery rate for a short stimulus.*

As was to be expected the maximum rate of recovery heat production, as represented by the height of the hump in the analysis, is higher for a longer stimulus and at a higher temperature. Expressed, however, as a fraction of the total initial heat, while preserving the difference for different temperatures, it does not seem to be consistently different for different durations of stimulus, at least to 12 sec. Table I summarizes

TABLE I.

No. of analysis	Temperature °C.	Duration of stimulus sec.	Maximum recovery heat rate, p.c. of total initial heat
1	10.5	4	6.7
2	10.5	4	5.0
3	11.0	12	5.3
4	10.5	4	6.0
5	10.5	2	5.0
Average	10.6		5.6
6	17.2	4	10.7
7	15.8	1	11.4
8	16.8	1	14.2
9	16.4	1	14.8
10	15.8	1	14.2
11	16.3	1	13.5
12	15.9	4	9.8
13	16.3	4	11.8
14	15.7	6	8.0
15	15.8	8	8.1
16	16.6	4	12.0
17	16.3	4	12.0
18	16.9	2	12.0
19	17.0	2	11.0
20	17.0	2	14.0
21	15.5	2	11.5
22	16.3	2	11.7
23	16.6	2	14.3
24	16.6	2	11.4
Average	16.4		12.2
25	20.5	4	14.0
26	20.0	4	15.7
27	19.3	4	16.0
28	20.5	1	18.6
Average	20.1		16.1

the analyses with respect to this point. The average maximum recovery heat production per second, as a percentage of the total initial heat, had the following values:

At about 10.5° C.	5.6 p.c.
At about 16.5° C.	12.2 p.c.
At about 20° C.	16.1 p.c.

These values will be used later in the discussion of the heat production of crab's nerve in nitrogen.

Hill [1929] states that the maximum rate of recovery heat production occurs almost immediately after the stimulus, and is about $6\frac{1}{2}$ p.c. of the initial heat per sec. at 15° C. The first statement depends upon the fact that he made his analysis in 5 or 6 sec. units and so missed the hump. The second does not agree well with our result; this, however, is partly accounted for by our taking the hump as the recovery maximum, which is of course higher than the average recovery heat rate over 5 or 6 sec. after the stimulus, as used by Hill. Moreover his "initial" heat, owing to the long interval of analysis, contained some recovery heat, which would tend to reduce the fraction calculated.

(3) *The magnitude of the initial heat.*

(a) *Initial heat per sec. of maximum stimulation per g. of moist nerve.*
The size of initial heat is naturally different for stimuli of different frequencies. It will serve no good purpose to record here all the values obtained at different frequencies under various conditions. Suffice it to collect the values of initial heat for fresh nerve at temperatures around 16.5° C. and stimulated at about 90 per sec.

TABLE II.

Exp. no.	1	2	3	4	5	6
Initial heat in microcal./g. × sec.				40.9	41.6	37.0	39.3	61.5	45.3
Exp. no.	7	8	9	10	11	Average
Initial heat in microcal./g. × sec.				65.5	38.1	53	37	43	45.7

About half the above results are from 1 sec. stimulus, the others from 2 or 4 sec. stimulus, in which case the heat rate during the first second is alone considered. We did not test in each case whether the stimulus was maximal or not. In cases where this was tested, increasing the voltage of the stimulus beyond that usually employed produced no significant increase of heat production. We feel safe to state in general that the strength of stimulus we employed could not have produced a response

smaller than 80 p.c. of the maximum. There is an additional factor, however, which must have tended to make the observed value of initial heat per g. low, and that is the probable presence of cut fibres in experiments in which dissection was very clean, or of tissue tags in those in which dissection was not so thorough.

(b) *The initial heat per single impulse.* From the intersection of the tangent to the frequency curve (Fig. 2, *A*) at zero time and the horizontal line representing the level of heat production corresponding to maximum frequency, it may be inferred that eighty isolated impulses would produce the same amount of heat as 1 sec. stimulation at maximum frequency at about 16.9° C. From the average initial heat per g. per sec. for stimuli of 90 per sec. recorded in the last section, the value for maximum frequency can be obtained by extrapolation on the frequency curve; it is 58.5×10^{-6} cal./g. sec. This number divided by 80 gives 0.73×10^{-6} cal./g. as the initial heat per isolated impulse at about 16.5° C. For frog's nerve Hill gives 0.26 microcal./g. and 0.067 microcal./g. as the values of initial heat per single isolated impulse at 0° C. and 20° C. respectively. Thus the initial heat due to a single isolated impulse of crab's nerve at about 16.5° C. is about three times as great as that of frog's nerve at 0° C. and at least ten times as great as at 20° C.

We have no data for calculating the impulse heat at lower temperatures with the same accuracy as for 16.5° C. From the size, however, of comparable records of 1 or 2 sec. stimuli at such low frequencies as 6, 10 or 16 per sec., given by nerves at 16° C. and at 10° C., we feel sure that the initial heat per single impulse is not smaller at 10° C. than at 16.5° C., but, if anything, greater.

It may be of interest to note that we have obtained with a single Zc galvanometer, without amplification, records of heat production due to five isolated impulses of crab's nerves, measuring as much as 5 mm. in height. With a good thermostat and amplification it should be possible to register and measure directly the heat production due to a single isolated impulse of crab's nerve.

(4) *The ratio of initial heat to total heat.*

Hill, in his original investigation [1929] of the heat production of crab's nerve, stated that the initial heat is about $2\frac{1}{4}$ p.c. of the total heat. We had in the course of our work three experiments regarding this point (Table III).

In determining the total heat lasting over half an hour or so, the difficulty lies in the creeping of the base line, and one often feels at a loss

TABLE III.

No. of exp.	Temp. °C.	Stimulus		Total heat 10 ⁻⁶ cal./g.	Initial heat 10 ⁻⁶ cal./g.	P.c. of total heat
		Duration sec.	Frequency shocks per sec.			
1	19.3	4	92.0	7330	145	2.0
2	20.0	4	92.0	15500	180	1.2
3	18.0	170	6.7	25800	718	2.0

as to how the latter should be drawn. Of the three experiments above, No. 1 was worst and No. 2 best in this respect. In No. 3 the duration of stimulus was 3 min., so there is a good deal of uncertainty in the estimation of initial heat as well. It is probable that Hill's value is somewhat too high, since his "initial" heat, determined by analysis in 5 or 6 sec. units, must have included some recovery heat.

(5) *Short stimuli in nitrogen.*

The distinctness of initial and recovery heats in crab's nerve offers a favourable opportunity for testing the question of their possible separation by means of oxygen want. The difficulty is that crab's nerve is incapable of standing any considerable amount of stimulation in nitrogen. Fig. 10 gives some idea of the rapidity with which it fails. In curve *A*, the four 4 sec. stimuli at 127 per sec. were all the stimulation given after introduction of nitrogen; similarly, in curve *B*, the total amount of stimulation in nitrogen was the five 4 sec. stimuli at 92 per sec. plotted. Yet in half an hour's time the nerve had failed completely. At rest, however, in nitrogen the nerve is able to keep its excitability much longer, and it is generally possible to obtain one or two good records in nitrogen for analysis.

Fig. 11 shows the record of a 4 sec. stimulus at 92 per sec. in nitrogen at 17° C. and its analysis. In this experiment nitrogen was introduced immediately after sucking out the sea water, and the record shown was taken 46 min. afterwards as the first thing in the experiment. It is considerably smaller than records usually are in oxygen, but its analysis is indistinguishable in shape. Not only are there (*a*) the usual large outburst of initial heat during the stimulus, (*b*) the abrupt fall when the stimulus ends, and (*c*) the slow rising of recovery heat to a maximum, but the ratio of the maximum recovery heat rate to the total initial heat, namely 12 p.c., is the same as the average for oxygen experiments at about this temperature. As in frog's nerve, therefore, oxygen want merely alters the size but not the course of heat formation due to stimulation.

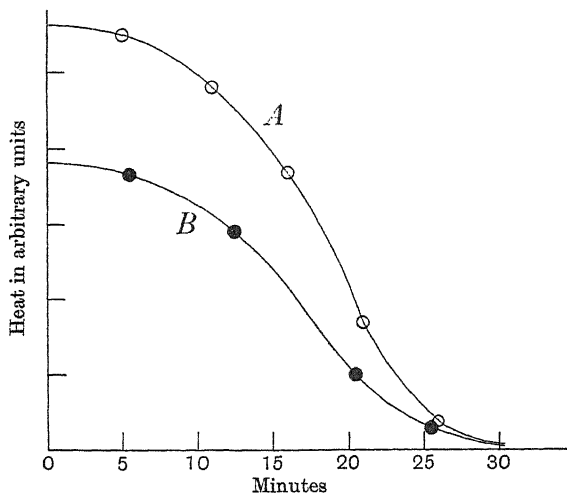


Fig. 10. The rapid failure of crab's nerve when stimulated in nitrogen. The points plotted represent the size of heat response to a 4 sec. stimulus at various times after the admission of nitrogen and include all the stimuli given in this gas. In both *A* and *B* complete failure occurred in about half an hour.

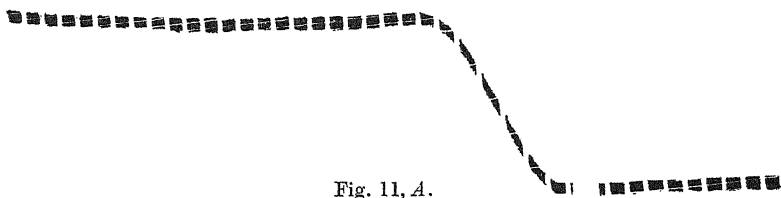


Fig. 11, *A*.

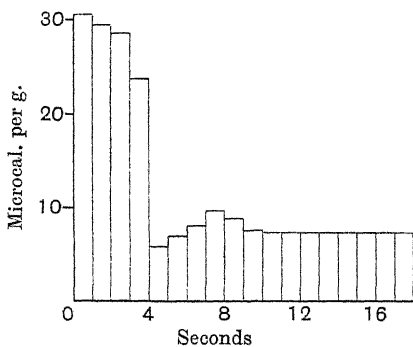


Fig. 11, *B*.

Fig. 11. *A*, record of a 4 sec. stimulus, 92 per sec., 46 min. after introduction of nitrogen, at 17° C., nerve having received no stimulation previously. *B*, analysis of same record. Though somewhat smaller in size it is otherwise indistinguishable from typical analyses in oxygen due to similar stimuli.

In this connection Furusawa's finding will naturally be recalled, that the "retention" of action potential disappears very much more rapidly in oxygen than in nitrogen; this disappearance is generally assumed to be associated with the recovery process, and so presumably with recovery heat production. A direct comparison, however, of his finding with our heat analysis we hesitate to make. In the first place he started observing his "retention" curves 1 min. after the stimulus at $\frac{1}{2}$ or 1 min. intervals, while our analysis was generally carried no further than 20 sec. In the second place his results were for stimuli as long as 20 sec., 50 make and 50 break shocks per sec., while the stimulus we gave in nitrogen was always 4 sec.

Cowan and Feng have recently repeated Furusawa's experiments, using 2 to 4 sec. stimuli and observing the "retention" curves starting 3 or 6 sec. from the beginning of the stimulus at 6 sec. intervals for a period of 8 to 10 min. Their curves plotting amount of "retention" against time from the beginning of stimulus from 1 min. onwards confirm Furusawa in that the oxygen curve always falls much more steeply. During the first half minute or so, however, there is hardly any difference between nitrogen and oxygen curves. Any ordinate of the curve in that time, expressed as a fraction of action potential generated by the stimulus (represented by the maximum deflection of the galvanometer), is the same in oxygen and in nitrogen. The fact, therefore, that we found the heat analysis in nitrogen to be entirely unchanged from that in oxygen, at least up to about 20 sec., is not in conflict with what is known of the electrical change of the nerve in the two gaseous media, even though a strict correlation between the recovery heat production and the restoration of the initial polarized state of the nerve be assumed¹.

¹ A strict relation between the two is not borne out by experimental results. Cowan and Feng observed that about 86 p.c. of the action potential or depolarization caused by a 2 sec. stimulus disappears at the end of 6 sec., in which time the recovery heat production due to the stimulus can hardly have been completed to the extent of more than 1 p.c. Further, the total disappearance of the retention of action potential after a short stimulus of 2 to 4 sec. requires less than 2 min. according to Levin [1927]. This is rather short and possibly complicated by the gradual drift of injury potential. Cowan and Feng, taking precautions to allow for such drift, found the time to be about 10 min. at about 17° C. Recovery heat production, however, after 2 to 4 sec. stimulus, is certainly not completed in less than 20 min. Clearly some mechanism must exist in the nerve which effects an immediate restoration, more or less complete, of its working condition after stimulation. While ultimately the energetic processes testified by the recovery heat production are perhaps responsible for the restoration of the original polarized state of the nerve after a stimulus, between such processes and the actual event of restoration there must be essential links whose nature so far eludes us.

It is possible that if the heat analysis were carried on for several minutes after the stimulus the divergence in nitrogen and oxygen would become apparent, just as in the case of action potential retention reported by Furusawa and confirmed by Cowan and Feng. Because of the technical difficulty, however, of working with nerves in nitrogen over longer periods we have not put this point to experimental test.

There are, however, conditions under which asphyxiation, while leaving the general course of heat production unaffected, does appear to change the quantitative relation between recovery heat rate and the total initial heat that went before. Fig. 12, *A* and *B*, are analyses of two

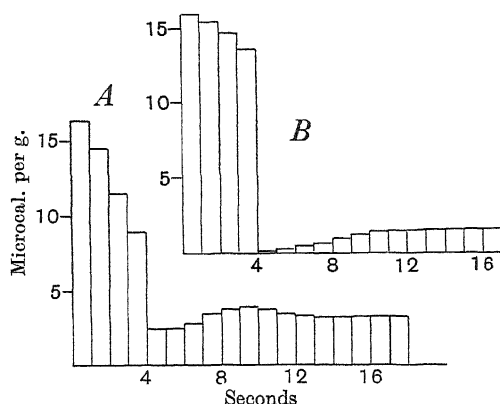


Fig. 12. Analyses of 4 sec. stimuli in nitrogen. *A*, 92 per sec. at 19.3° C., *B*, 44 per sec. at 11.0° C. In both cases the nerves had been partially exhausted previously. Note that the maximum recovery heat relatively to the total initial heat preceding is much lower in these two instances than usual for responses in oxygen.

4 sec. stimuli in nitrogen from two separate experiments at 19.3° C. and 11.0° C. respectively. Both are from partially exhausted nerves. Before *A* three 4 sec. stimuli in oxygen and another three 4 sec. stimuli after the introduction of nitrogen had been taken. *B* was the first stimulus 1 hour after the admission of nitrogen, but before the introduction of nitrogen the nerve had received five 4 sec. stimuli and seventeen 2 sec. stimuli at various frequencies. The maximum recovery heat rate in *A* was 8.5 p.c. of the total initial heat preceding as against 16 p.c., the average value for stimuli in oxygen at a similar temperature. The maximum was also reached somewhat later in time. In *B* the recovery process was so slow that the maximum did not seem to be quite reached even at the thirteenth second after the stimulus. Assuming the highest recovery rate, at the

end of the analysis shown, to be the maximum, it is about 3.1 p.c. of total initial heat, while 5.8 p.c. is the average for oxygen responses at the same temperature. The oxygen reserve of such partially exhausted nerves must be rather low. It is conceivable that the recovery heat rate, while primarily depending upon the amount of initial breakdown, adjusts itself somehow to the existing level of oxygen reserve, and that a depleted oxygen reserve affects the initial and recovery heat production somewhat disproportionately, the latter being relatively more affected.

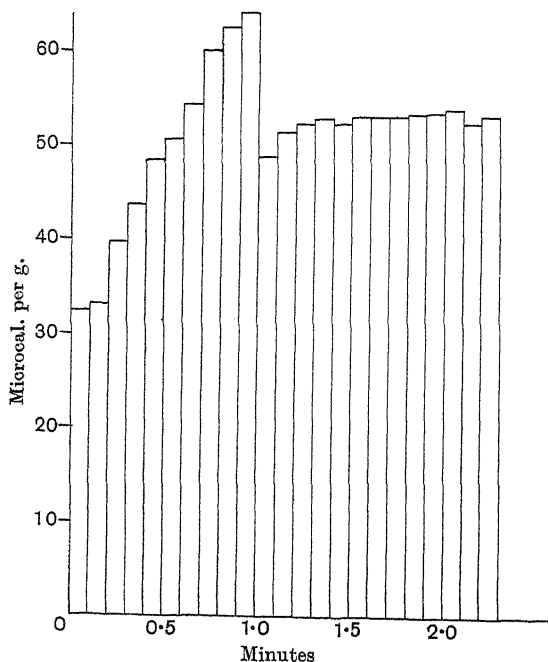


Fig. 13. Analysis of heat from 1 min. stimulus, 6.7 per sec. at 10.5° C., showing that at sufficiently low frequencies crab's nerve may be stimulated as long as 1 min. without failure.

PROLONGED STIMULATION.

Crab's nerve, while very fatigable, is not incapable of prolonged activity, provided it be stimulated at a sufficiently low frequency. In Fig. 13 is the analysis of a 1 min. stimulus at 6.7 per sec. The analysis was done by 6 sec. units with zero remainders. No attempt was made to smooth out the slight oscillations. It is clear that no significant failure occurs during 1 min. stimulation at this frequency. In Fig. 14 is shown (A)

a 91 min. stimulus at 4.3 per sec. and (*B*) a 130 min. at 1.2 per sec. In *A*, although a certain amount of failure is obvious in the course of stimulation, it is not much. In *B* no serious failure is discernible after 2 hours 10 min. continuous stimulation, except for the rather uncertain creep of the base line. What failure there was may well have been due to spontaneous deterioration of the nerve rather than to stimulation. A steady state of heat production is thus apparently possible in crab's nerve if

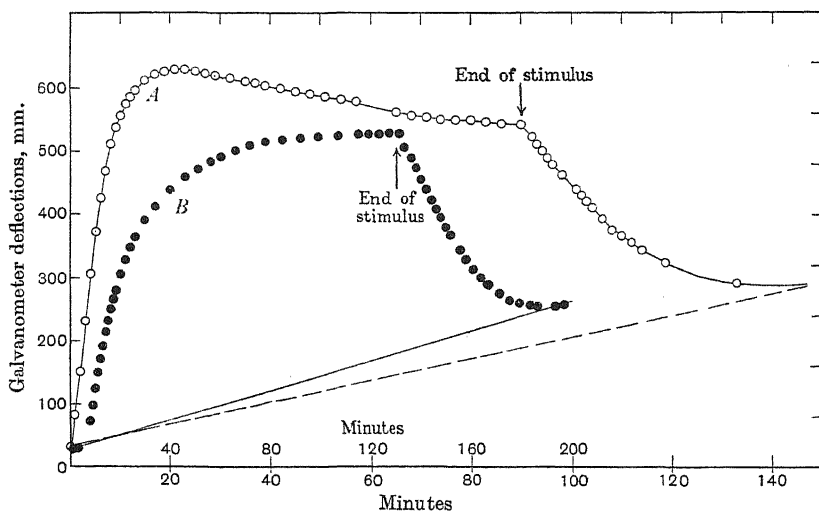


Fig. 14. Galvanometer deflections on scale for *A*, 91 min. continuous stimulation at 4.3 per sec.; *B*, 2 hr. 10 min. continuous stimulation at 1.2 per sec.; both at 19.5° C. Abscissa, inner scale for (*B*), outer for (*A*); ordinate, same for both. It is evident that crab's nerve is capable of keeping up prolonged activity at very low frequencies.

stimulated at the proper frequency, and the natural capacity of crab's muscle for prolonged steady contraction must depend upon the fact that, after a short preliminary period during which a high frequency is necessary, one or two impulses per second coming from its nerve are sufficient to keep it in tetanic contraction.

It is interesting to calculate the ratio of total heat to initial heat in such an extremely prolonged stimulus as shown in Fig. 14, *B*. Since there is no definite sign of failure in the course of stimulation, it may be assumed that each stimulus continues to call forth a full-sized impulse. From a preceding section, the initial heat per single isolated impulse is 0.73 microcal./g. At a frequency of 1.2 per sec. the impulse set up may be considered as practically isolated. Hence it may be calculated that the

total initial heat in the 130 min. stimulation is 6830×10^{-6} cal./g. The total heat can be estimated from the total area under the curve and above the base line, as drawn in Fig. 14, *B*. It is 518,000 microcal./g. So the ratio of the total heat to initial heat is 76. While this number is only an estimate, it is remarkable that it comes to be of the same order as that more directly determined in a short stimulus.

THE RESTING HEAT RATE OF CRAB'S NERVE.

Several experiments were made to estimate the resting heat production of crab's nerve in oxygen and in nitrogen and when poisoned by iodoacetic acid. Only a thermostat at 20.7°C . was available and all observations were made at that temperature. To avoid the complication of a vapour pressure effect, nerves were always soaked $2\frac{1}{2}$ to 3 hours in sea water in the thermopile chamber before taking observations; the thermopile, however, was generally kept in a bath of about 14°C . during the first 2 hours' soaking, then transferred to the thermostat at 20.7°C . This procedure was adopted for fear that the deterioration of crab's nerve at the higher temperature might be too rapid, and to increase the probability that the heat rate observed soon after sucking out sea water from the thermopile chamber might be representative of fresh nerve. No test of the condition of the nerves by stimulation was carried out. Following is a summary of the results on normal nerve:

TABLE IV. Resting heat rate in cal./g. hour.

No. of exp.	In oxygen	After admission of nitrogen (hours)					
		1	2	3	4	5	6
1	0.72	0.23	0.15	—	—	—	—
2	0.83	0.30	0.22	0.17	0.13	0.11	0.10
3	0.65	0.27	0.19	0.14	0.12	—	—
4	0.53	—	0.13	0.17	—	—	—
5	0.74	—	—	—	—	—	—
Average	0.69	—	—	—	—	—	—

According to Meyerhof and Schulz, the oxygen consumption of crab's nerve at rest at 16°C . is 0.8 c.c. per g. of dry weight per hour, or about 96 c.mm. per g. of moist tissue per hour, assuming a water content of 88 p.c. for these nerves. This may be considered to be equal to a heat liberation at the rate of 0.48 cal./g. hour. Assuming a $Q_{10} = 2.5$, the corresponding value at 20.7°C . is 0.74 cal./g. hour, very near to our average value of 0.69 cal./g. hour found by direct measurement.

On the basis of the recent measurement by Beresina [1932] the

resting heat rate of crab's nerve in oxygen is about three times as great as that of frog's nerve under similar conditions.

The resting heat production of crab's nerve drops relatively very sharply on replacing oxygen with nitrogen. After 1 hour it is reduced to about 25 p.c. of the oxygen value; after 2 hours to under 20 p.c.; then occasionally remaining approximately constant or more often increasing slowly. In the case of frog's nerve it takes $2\frac{1}{2}$ hours to reduce the resting heat rate in nitrogen to 20 to 25 p.c. of that in oxygen; once reduced to this lower level it apparently remains constant for hours [Beresina, 1932]. Crab's nerves contrast with frog's nerves in respect to both these features. Perhaps the more rapid fall of the resting heat rate of crab's nerve in nitrogen may be related to its earlier onset of inexcitability when subjected to oxygen want, particularly when it receives at the same time any amount of stimulation. It seems that the oxygen reserve in crab's nerve is smaller: perhaps not per g. of nerve but relatively to the fact that the resting heat rate of crab's nerve is about three times as large as that of frog's and that the total heat liberation (initial plus recovery) due to a short stimulus more than thirty times as great. The fact that a constant level of resting heat production of crab's nerve in nitrogen is seldom maintained probably means that the rate of glycolysis which is presumably largely responsible for the anaerobic resting heat begins to fall earlier and more rapidly in this nerve than in frog's.

Holmes [1929] has investigated the change of glycogen content and lactic acid production of crab's nerve resting in oxygen and in nitrogen. As emphasized, however, by the author himself, his results show extreme variations, and no significant average could be taken. Correlation of his results and ours, therefore, is hardly possible.

The effect of iodoacetic acid on crab's nerve is far more pronounced than on frog's. The latter requires 2 hours or longer soaking in 0.4 p.c. iodoacetate to produce conspicuous poisoning [Feng, 1932], while 40 min. soaking in 0.05 p.c. iodoacetate for the former already considerably diminishes its response to a short stimulus, and very greatly decreases its ability to stand further stimulation. For resting heat study, where soaking cannot safely be less than 2 hours because of the vapour pressure complication, two concentrations of iodoacetate were tried, 1/4000 and 1/8000 in sea water. The stronger concentration in all the four experiments made with it caused the initial level of resting heat rate in oxygen to be somewhat lower than in normal nerves; the more striking effect, however, was that even this lower level was not maintained but progressively diminished to practically nothing or became negative in 3 to 5

hours. With the weaker concentration also one generally found a slightly lower initial rate of heat production which, however, remained approximately steady at least for 1 hour or so. The average of four experiments gave 0.56 cal./g. hour as the resting heat rate in oxygen of crab's nerves poisoned by 1/8000 iodoacetate. On introducing nitrogen the fall of heat rate was much quicker than in normal nerves. In about 2 hours' time it was practically nothing, and became negative when observation was further continued. Here, again, we have negative heat production. While it is not difficult to conceive that, in a strongly poisoned nerve, where both respiration and glycolysis are completely or nearly inhibited, the resting heat production as observed may be nothing, one cannot easily imagine how negative heat production could occur, apart from technical complications, *e.g.* of a galvanic effect due to imperfect insulation. The point is not yet clear.

DISCUSSION.

Hill showed that the course of the recovery heat production in frog's nerve can be represented by a double exponential of the form

$$Y = Ae^{-at} + Be^{-bt}.$$

The application of such an equation to the recovery heat production of crab's nerve is out of the question, for here the recovery heat rate, instead of starting out from its highest value immediately after the passing of an impulse, as required by the equation, begins low and gradually rises to a maximum. Such a time course would require for its representation an equation of the type

$$Y = Ae^{-at} - Be^{-bt}.$$

Like many natural processes, indeed like the recovery process in muscle [Hartree, 1932], the recovery process in nerve gradually works up to its maximum and then declines.

One of the more striking characteristics of crab's nerve is its great susceptibility to fatigue when stimulated even at very moderate frequencies. While no answer is possible to the question why frog's nerve is so resistant to fatigue while crab's nerve is so susceptible, it may be relevant to note that the recovery heat production after a stimulus starts out at its maximum rate in frog's nerve, and takes a much quicker course than in crab's nerve, in which the maximum recovery rate is not reached until some seconds after the stimulus is over. It is possible that fatigability is due to slow recovery, testified by a slower recovery heat production. At any rate at lower temperatures where recovery is further

slowed down, the fatigability of crab's nerve becomes more pronounced. At 0° C. even frog's nerve shows evident signs of fatigability in a stimulus as short as 16 sec. at 30 to 40 per sec. [Hill, 1932].

Now fatigability is also a characteristic of the central nervous system. May we not suspect conversely that, if we could get a workable preparation of grey matter for neurothermic study, we could find that here too the course of the recovery heat production after a stimulus was slow, very much more similar to that in crab's than in frog's nerve?

Recently there has appeared an investigation by Hoffmann, Holzlohner and Leegaard [1932] on the heat production of the surviving spinal cord of the frog. They report that for durations of stimulus from 1.3 to 19.5 sec. at 21 to 22° C. the total heat production per sec. of stimulation per g. wet weight of the spinal cord is 580 to 2324 microcal.; values comparable to those given by crab's nerves. According to them, however, all this heat was completed within 2 to 5.4 min. Further they present analyses showing that the heat rate after the stimulus may be even somewhat greater than during the stimulus; that in a stimulus of about 5 sec. the average heat rate in the 15 sec. or so following the stimulus is nearly as high as that during the stimulus. Unfortunately they do not discuss either of these unusual features or the possibility of after discharge following the stimulus. Their work, as it stands, does not support any correlation between fatigability and low rate of recovery heat production in the case of the central nervous system such as seems to exist in crab's nerve.

SUMMARY.

1. The heat production of crab's limb nerve has been more fully investigated than in Hill's original work on the subject.

2. The optimal stimulus for this nerve at about 17° C. has an RF (resistance in ohms \times capacity in μF , which is proportional to time of discharge) of about 1200, which is six times as great as for frog's nerve.

3. The effect of frequency of stimulation has been determined, the maximum response being obtained at a frequency (at about 16.9° C.) in the neighbourhood of 200 per sec.

4. Analysis of photographic records of heat production due to short stimuli in oxygen and nitrogen was carried out in 1 sec. units. The separation of initial and recovery phases is very distinct; the maximum of recovery heat rate is not reached until some seconds after the stimulus is over, so that there is always a very abrupt fall of heat rate at the end of a stimulus and always a "hump" in the recovery part of the analysis.

5. The course of the heat production of fresh crustacean nerve in nitrogen is exactly the same as in oxygen. Nerves, however, which have previously been partially exhausted have a lower recovery heat rate, relatively to the total initial heat going before.

6. The initial heat due to nearly maximal stimulation both with respect to strength and frequency is found to be 58×10^{-6} cal./g. sec.: this is $1\frac{1}{2}$ to $2\frac{1}{2}$ p.c. of the total heat (initial plus recovery).

7. The initial heat per single isolated impulse is about 0.73 micro-cal./g. at 16.5°C .

8. While stimulated even at frequencies only about a quarter or so of the maximum to which it will respond, crab's nerve is shown to be very fatigable; it is quite capable, however, of maintaining prolonged activity if the frequency of stimulation is very low. It shows no distinct failure when stimulated at 1.2 per sec. for as long as 2 hours.

9. The resting heat production of crab's nerve in oxygen at 20.7°C . is about 0.69 cal./g. hour, agreeing satisfactorily with the oxygen consumption measurement of Meyerhof and Schulz. Some observations of resting heat production of nerves in nitrogen and when poisoned by iodoacetic acid are recorded.

The authors owe deep gratitude to Prof. A. V. Hill who suggested this work and whose aid and encouragement kept it going. To Dr E. J. Allen and the staff of the Plymouth Laboratory they wish to express their sincere thanks for kind hospitality which made their stay so enjoyable; to Mr S. L. Cowan they are indebted for much willing assistance as well as pleasant discussion in the course of their work.

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THE LOCALIZATION OF THE ACTION OF DRUGS
ON THE PULMONARY VESSELS OF
DOGS AND CATS.

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SINCE previous studies of the effect of drugs on the pulmonary vessels have been reviewed by Wiggers [1921], Tigerstedt [1923] and Daly [1932], it is not necessary for us to present a connected historical survey. We will discuss earlier work only in so far as it bears directly on our own experiments, which were devised with the chief object of obtaining information regarding the site of action on the pulmonary circulation of various pharmacologically active substances which are found in tissue extracts.

In early work on this subject the assumption was usually made that any observed effects of drugs on the resistance of the pulmonary vessels to the flow of blood were due to an action on the pulmonary arterioles. The suggestion that changes in the tone of the pulmonary veins might appreciably affect the resistance originated partly from the study of the action of drugs on isolated veins [Inchley, 1923, 1926; Franklin, 1932, who gives other references], and partly from observations of the lung volume when the circulation was intact [Luisada, 1928; Mautner and Pick, 1929]. Mautner and Pick have shown that the blood flow through the portal system of the dog is much influenced by the resistance offered by the hepatic veins under the action of histamine, and they give evidence suggesting that histamine also causes the pulmonary veins to constrict.

The conclusions of Mautner and Pick regarding the portal circulation have been confirmed and extended in an investigation carried out in this laboratory [Bauer, Dale, Poulsson and Richards, 1932]. The present work was undertaken with the object of applying similar methods to the study of the behaviour of the pulmonary vessels. It was hoped that fresh light might be thrown on the importance of the pulmonary veins by

recording the changes in the lung volume as well as other effects during perfusion.

The most widely used method of studying the effect of drugs on the pulmonary vessels is that introduced by Brodie and Dixon [1904], in which the lungs are perfused with blood under constant pressure and the effect on the outflow is recorded. Under these conditions the effect of a vaso-constrictor drug is seen in a diminution of outflow, and some information regarding the main site of the effect may be obtained by recording the volume of the lung. If the effect is predominantly on the inflow to the lung the volume of the lung will be diminished; if it is predominantly on the outflow the volume will be increased. Analogous conclusions may also be drawn regarding the action of vaso-dilator drugs.

In another less commonly used method of perfusing the lungs, the pulmonary artery is connected directly to the perfusion pump, so that the rate of flow is approximately constant and the effects of the drugs are seen in changes in the pressure in the pulmonary artery. If the pump were completely rigid these changes of pressure would not affect its output. Under these conditions, a vaso-motor drug would have practically no effect on the volume of the lung, except in so far as it acted on the veins.

The apparatus which we have used was so devised that the perfusion could be carried out either under conditions of approximately constant pressure, or under conditions of approximately constant flow. In the former case, changes in the volume of the lung were partly due to changes in the resistance to inflow and partly due to changes in the resistance to outflow. In the latter, the effect on the volume of changes in the resistance to inflow was largely eliminated, and effects due to changes in the resistance to outflow were better shown. By changing repeatedly from one method of perfusion to the other, it has been possible further to test the conclusions suggested by the observed effects of drugs. The two methods of perfusion are easily distinguished in the figures, since when the pump is connected directly to the artery the record of the inflow pressure shows large oscillations with the rhythm of the pump, which are absent when the other method of perfusion is used.

The effects shown in our records of lung volume are presumably due to changes in the volume of blood in the capillaries, and it is possible that they are caused in some cases by changes in the tone of these vessels, though we know of no evidence that such changes can occur in the lungs. Constriction of the capillaries would produce an increase in resistance and a decrease in the lung volume, and would thus be indistinguishable in our experiments from constriction of the arterioles, except in so far as the

constriction of capillaries might cause a transient increase in outflow owing to their large blood capacity. When such a combination of effects has occurred, we have been unable to decide whether the effect should be attributed to an action on the arterioles or on the capillaries, and we have been content to consider the two kinds of vessel together and to attribute such effects to an action on the "inflow."

METHODS.

The animals (dogs and cats) were anaesthetized with ether and bled through the carotid artery. The blood was either defibrinated or collected in a bowl containing heparine. In experiments with cats, about 50 c.c. of Ringer's solution were usually injected into the jugular vein, so that sufficient blood to fill the apparatus (at least 120 c.c.) was obtained from a single animal. The lungs were then removed from the animal, the arterial cannula was inserted in the pulmonary artery through the wall of the right ventricle, and the venous cannula was inserted through the left auricular appendix as deeply as possible without obstructing the flow, so as to reduce to a minimum possible passive changes in the volume of the auricle. A thread was tied round the heart at the base of the ventricles, in order to prevent blood from escaping from the left auricle through the mitral valve. The bronchial vessels were not perfused.

The lungs were enclosed in the plethysmograph described by Bauer, Dale, Poulsson and Richards [1932], and their volume was recorded by means of a Brodie bellows [1902] attached to this plethysmograph. A tube, which was tied into the trachea, passed to the outside of the plethysmograph and was closed with a clip. It is known that the distension of the lungs by negative pressure applied to the pleural surface decreases the resistance of the pulmonary vascular bed [Daly, 1930], but if the distension is produced by a positive pressure in the trachea the resistance may be increased. As it was desirable to reduce the proportion of the resistance due to simple mechanical factors, the lungs were perfused in an almost completely deflated condition.

Conditions resembling more closely those occurring in the intact animal would have been obtained if the lungs had been rhythmically inflated during the experiment. Under these conditions, however, it would have been difficult to distinguish effects on the average volume due to constriction of the bronchi from those due to changes in the blood content of the lungs. We believe that, under the conditions we have chosen, it is unlikely that changes in the tone of the bronchi will affect the record.

In order to convince ourselves that the changes which we were recording did really represent changes in the blood content of the lung, we have performed a control experiment in which we used an elegant method introduced by Daly [1930]. Daly recorded changes in the volume of blood in perfused lungs by recording the corresponding changes in the volume of blood outside the lungs in the apparatus. In most of our experiments we did not use this method because of the difficulty of combining it with a record of the outflow. In our control experiment, the pulmonary artery of a dog was connected directly to the pump and the outflow was not recorded. The plethysmographic record was compared with the record of the volume of blood in the venous reservoir. It was found that occlusion of the inflow or outflow, or the injection of adrenaline histamine or acetylcholine, produced similar but opposite, immediate effects on the two records. The plethysmographic record was slightly less sensitive than the other record and was more liable to show changes unconnected with the injection of drugs, which were probably due to small changes in the temperature of the plethysmograph. We concluded that records obtained in this way would give us a reliable indication of the main site of action of drugs, and enable us to record the rate of outflow from the veins as well as the volume of blood in the lung.

A bath containing water thermostatically kept at 40° was placed about 15–25 cm. above the level of the lungs. This bath contained a spiral glass coil, a pump [Dale and Schuster, 1928] and an arterial reservoir for blood (Fig. 1). Blood from the venous reservoir first passed through the coil and then the pump. After leaving the pump it could be shunted by means of artery forceps applied to rubber connections, so that it either passed directly into the pulmonary artery or else into the arterial reservoir and thence to the pulmonary artery. The level of the blood in the arterial reservoir was kept constant by means of an overflow tube to the venous reservoir. A vertical glass tube was connected to the arterial supply by means of a T-piece. The height to which the blood rose in this tube gave a measure of the arterial pressure, and this was recorded by means of a small Brodie bellows connected to the upper end of the vertical tube. The venous outflow was recorded by the method described by Gaddum [1929], and the blood was collected in a venous reservoir which was open to the air. The outflow recorder was at about the same level as the auricle.

The apparatus was originally designed for use with cats, and our records show that when cats' lungs were perfused the apparatus was effective in maintaining either the perfusion pressure, or the rate of flow,

Two types of difficulty were encountered in experiments with cats. In the first place, it sometimes happened that the vessels rapidly acquired such a marked tone that the blood almost ceased to flow. In such cases, the addition of adrenaline to the blood was found to relax the tone and to allow the experiment to be completed [cf. Löhr, 1923]. In the second place, the plethysmographic record frequently showed a steady increase in lung volume, which was so marked as to prevent records being taken until more than an hour after the perfusion started. This change was apparently due to the use of defibrinated blood. When heparine was used, the lung reached a steady volume much more rapidly. These difficulties were not encountered in experiments on dogs' lungs, and in this case no difference was noticed between the results obtained with defibrinated and heparinized blood.

The drugs were injected into the rubber tubing leading to the arterial cannula. The volume of fluid injected was between 0.05 and 0.2 c.c. The solution of adrenaline used in most of the experiments contained chlore-tone, but a few control experiments showed that all the effects recorded were also produced by a sample of the base prepared synthetically and dissolved by the aid of HCl. Histamine was used in the form of the acid phosphate, but the doses are given in terms of the base. The doses of all other substances are given in terms of the total weight of the salt used to prepare the solutions for injection.

We wish to express our thanks to Dr K. Lohmann who very kindly supplied us with the specimens of muscle adenylic acid and adenylypyrophosphate which we have used. The latter was in the form of the barium salt. This was dissolved with the aid of a small quantity of HCl. An excess of sodium sulphate was added to precipitate the barium and the solution was then centrifuged. The supernatant fluid was neutralized before use with NaOH.

RESULTS.

Adrenaline.

Dogs. In confirmation of much previous work, we find that adrenaline normally increases the resistance of the dog's pulmonary vessels to the flow of the blood. This effect is accompanied, as shown in Fig. 2 *a*, by a fall in the lung volume, and is thus mainly due to the constriction of the inflow. This conclusion is confirmed by the fact, shown in Fig. 2 *b*, that when the rate of inflow is made more nearly constant by connecting the pulmonary artery directly to the pump the effect on the volume is diminished. In two experiments out of ten, the substitution of a direct

perfusion by the pump for the perfusion under constant pressure actually caused a reversal of the effect on the volume, which was now increased under the action of adrenaline (cf. Fig. 5). Though the main action of adrenaline in this experiment was on the inflow, the drug appeared therefore to have also a constrictor action on the outflow.

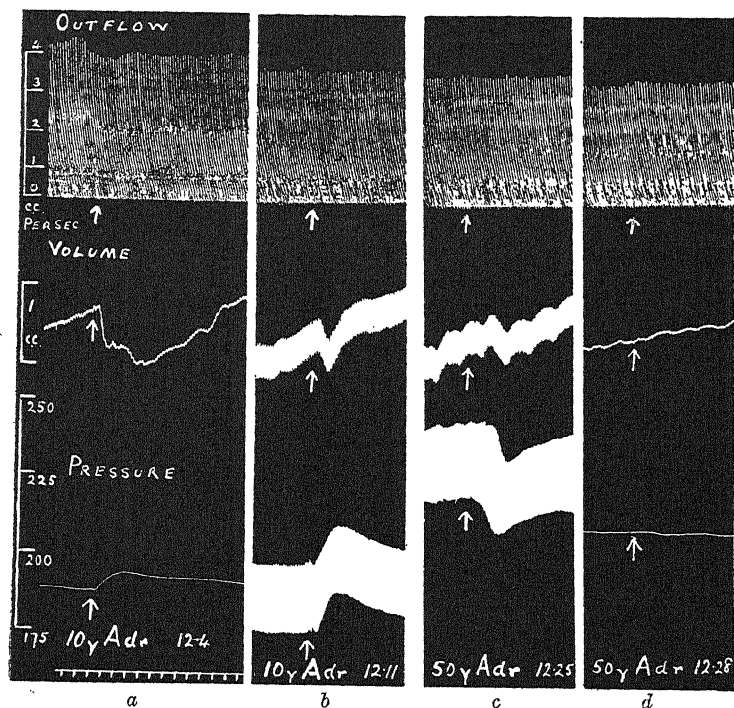


Fig. 2. Dog. Adrenaline (+chloretone). Outflow. Lung volume. Perfusion pressure (mm. of blood). At 12.16, 1 mg. of ergotoxine ethane sulphonate was added to the blood in the venous reservoir.

Fig. 3 shows another effect which adrenaline sometimes produced. In this record, the second injection of 25 γ of adrenaline is seen to cause a slight rise in the perfusion pressure. This would not be sufficient appreciably to affect the output of the pump, but the effect, if any, would be to cause a diminution of the rate of flow of blood into the lungs. Nevertheless, the rate of outflow was definitely increased. Under these circumstances the effect on the volume of the lung was inevitably a fall. This increase of outflow was clearly shown when the pump was connected

directly to the pulmonary artery (Fig. 3 *b*), but not when the lungs were perfused at constant pressure (Fig. 3 *a*). This was presumably because the rate of inflow fell in the latter case, owing to increase of the inflow resistance, while it was maintained practically constant in the former case. Adrenaline certainly produced a restriction of the inflow in this experiment, but the record of the rate of outflow shows that it had other effects as well. We do not know whether this increase of outflow was due to dilatation of the pulmonary veins letting the blood flow out, or to constriction of the capillaries forcing it out.

The effect which has just been described is the only evidence we have obtained which might be interpreted as indicating that a drug was having an action on the outflow contrary to its action on the inflow. When this phenomenon was first observed, it seemed possible that adrenaline had a specific action in releasing blood from the pulmonary circulation, just as it has been found to release blood from the portal circulation. Fig. 3 shows the most striking example of this phenomenon which we have seen. This effect was only seen in five out of ten experiments, and, as has already been mentioned, two experiments provided definite evidence that adrenaline was constricting the pulmonary veins. We have not been able to determine what factors govern

the direction of the action of adrenaline on the pulmonary veins.

The constrictor effect of adrenaline is reversed by ergotoxine. In the experiment shown in Fig. 2, 1 mg. of ergotoxine ethane sulphonate was added at 12.16 to the blood in the venous reservoir. This produced a reversal of the effect of adrenaline on the perfusion pressure, while the direction of the effect on the volume was unaltered. The main effect of this dose of adrenaline was now dilator, and, since it still produced a fall in the lung volume, the effect was mainly on the outflow. This conclusion is supported by the fact that, after ergotoxine, the effect on the volume was best shown when the lungs were connected directly to the pump, so that the rate of flow of blood into the lungs was kept nearly constant.

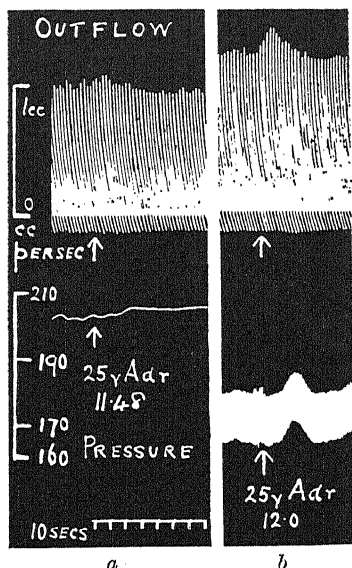


Fig. 3. Dog. Adrenaline (chlorotone-free). Outflow. Perfusion pressure (mm. of blood).

When the lung was perfused at constant pressure, the increase in the rate of outflow, which was slight but just visible on the outflow record, was compensated by an increased rate of inflow, so that no effect on the volume was produced. When the lungs were connected directly to the pump, this increase in the rate of inflow could not occur.

In another experiment the vaso-dilator action of adrenaline after ergotoxine was accompanied by an increase in the volume of the lung, which was most marked when the perfusion was carried out at constant pressure. In this experiment the dilatation must have been mostly due to an effect on the inflow.

Cats. It is well known that adrenaline may, under different conditions, produce either constriction or dilatation of the pulmonary vessels of the cat. The factors controlling the direction of the effect have been studied by Tribe [1914], and we are able, in general, to confirm her conclusions. In the early part of the experiments, the injection of small doses of adrenaline has invariably caused a large and prolonged vaso-dilatation. This effect is shown in Fig. 4. When the lungs were perfused in this experiment at an approximately constant pressure (Fig. 4 *a*), the injection of 2γ of chloretone-free adrenaline caused an increase in the rate of outflow and no effect on the volume of the lung. When the artery was connected directly to the pump (Fig. 4 *b*), the same injection caused a fall in the perfusion pressure and a small fall in lung volume. The record of outflow shows that the change in the perfusion pressure was not sufficient appreciably to affect the output of the pump. It is clear from the effect of this second injection that adrenaline may produce dilatation of the pulmonary venules in the cat. The fact that adrenaline had no effect on the volume when the perfusion was carried out at constant pressure shows that the pulmonary arterioles were also dilated, and that the increase in the rate of outflow from the lungs was in that case exactly compensated by an increase in the rate of inflow. When the artery was connected directly to the pump, no such increase in the rate of inflow could occur, and the volume of the lungs accordingly fell. Results similar to those shown in Fig. 4 have usually been obtained, but, in some experiments in which adrenaline produced similar evidence of vaso-dilatation by both methods of perfusion, it had no definite effect on the volume of the lung, even when the artery was connected directly to the pump.

In our first experiments, carried out in October, the vaso-dilator action of adrenaline was only seen in the early part of the experiment. The injection of a large dose of adrenaline at this stage caused a very large dilatation. In one experiment, for example, the injection of 0.1 mg. of

adrenaline increased the flow to four or five times its original value, and the vessels never recovered their original tone. In these first experiments, about an hour after the perfusion had started, the injection of small doses of adrenaline ceased to have any effect, and the injection of larger doses (20γ – 200γ) caused vaso-constriction. In later experiments, carried out in March, the vaso-dilator action was much more persistent, and, in order to obtain vaso-constriction, it was necessary to use chloretone-free adrenaline,

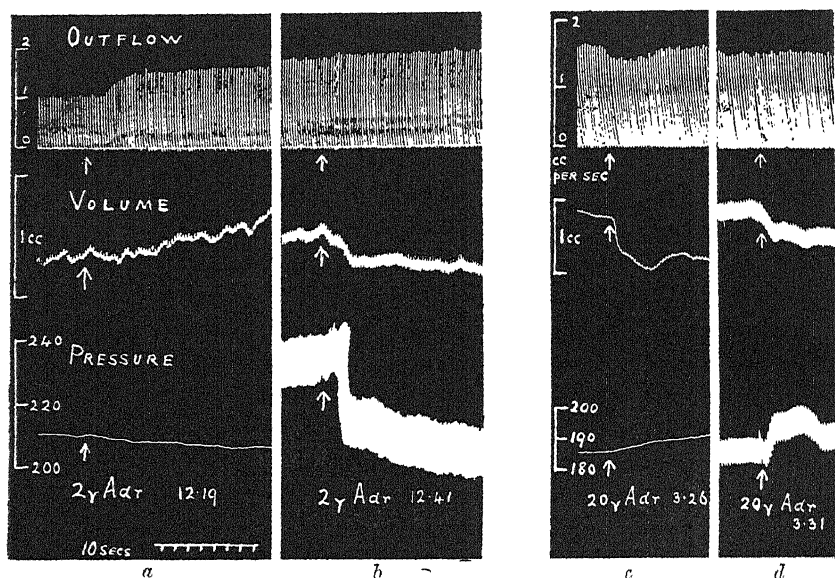


Fig. 4. Cat. Adrenaline (chloretone-free) in two different experiments. Outflow (c.c. per sec.). Lung volume. Perfusion pressure (mm. of blood).

so that the constrictor effect of the adrenaline should not be masked by the feeble dilator effect of the chloretone. Fig. 4 *c, d* show that this action of adrenaline was mostly due to constriction of the inflow. It was accompanied by a fall in the volume of the lung, which was most marked when the perfusion was carried out at constant pressure (Fig. 4 *c*). When the artery was connected directly to the pump the rate of inflow was artificially maintained almost constant and comparatively little change in lung volume occurred (Fig. 4 *d*). It is possible that the small decrease in volume which did occur under these conditions was partly due to dilatation of the venules.

Histamine.

In their original description of the effect of histamine in increasing the resistance of the pulmonary vessels, Dale and Laidlaw [1910] assumed that the effect was due to an action of the drug on the pulmonary arterioles. Inchley [1923], however, devised a method of perfusing arteries and veins separately with Ringer's solution, and showed that they were both constricted by histamine. Later [Inchley, 1926] he found that isolated

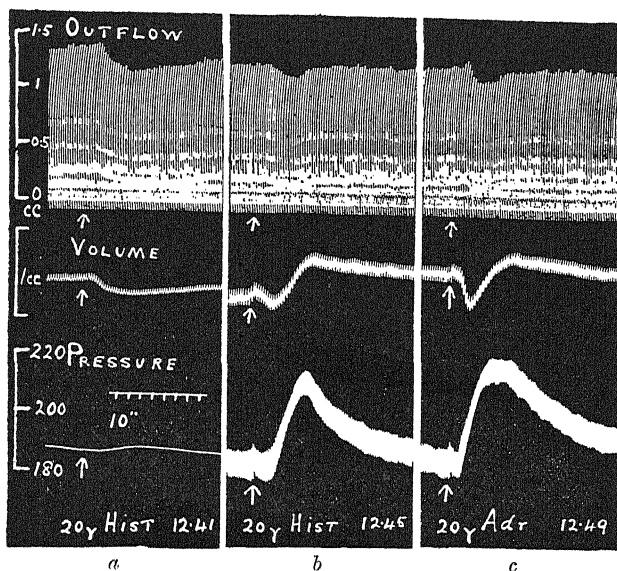


Fig. 5. Dog. Histamine and adrenaline (chloretone-free). Outflow (c.c. per sec.). Lung volume. Perfusion pressure (mm. of blood).

veins in general, and the pulmonary veins in particular, were more sensitive to histamine than isolated arteries, and concluded that the effects of histamine were mainly due to an action on the veins. This view was also held by Mautner [1923] and was supported by experiments in which the lung volume of dogs with intact circulation was recorded, together with the pressures in the pulmonary artery and in the left auricle [Luisada, 1928; Mautner and Pick, 1929].

In our experiments, histamine has invariably produced vaso-constriction, both in cats and dogs. When the perfusion was carried out at constant pressure, the fall in blood flow was usually accompanied by a fall in lung volume, and was thus mainly due to constriction of the inflow.

When the artery was connected directly to the pump, this fall in lung volume was followed by a rise which was probably due to the constriction of the outflow. These effects are shown in Fig. 5, taken from an experiment on a dog, and in Fig. 6, taken from an experiment on a cat. In some experiments, larger doses of histamine produced a very large and prolonged rise in lung volume. This effect was not reversible and was probably due to oedema. In the experiments from which tracings are given, the volume

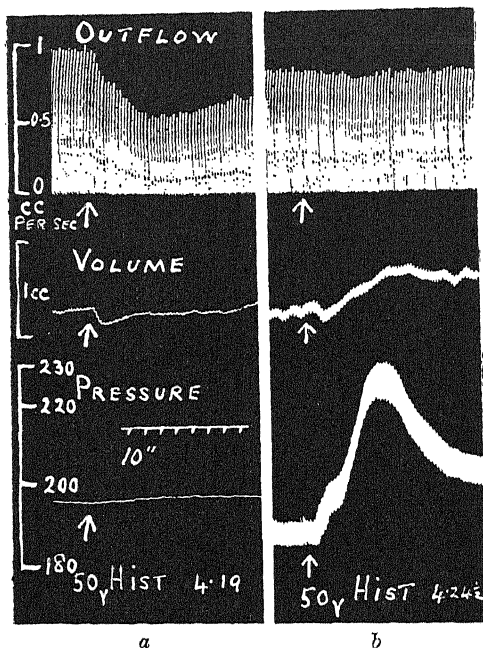


Fig. 6. Cat. Histamine. Outflow, Lung volume.
Perfusion pressure (mm. of blood).

showed a tendency to return to its original level, and, though it is possible that histamine caused some oedema in these experiments, the rise in volume cannot have been entirely due to this, and must have been caused, at least in part, by some more easily reversible change.

In Fig. 6 *b* the conclusion that histamine produced constriction of two separate structures is confirmed by the double nature of the rise in perfusion pressure. The first effect of the drug was constriction of the inflow, which caused a rise of perfusion pressure and a small fall of volume. This was rapidly followed by constriction of the outflow, which caused a further rise of perfusion pressure and a simultaneous rise in lung volume. In the

same way the record in Fig. 6 *a* which shows the rate of outflow when the lung was perfused at constant pressure also gives an indication that the drug was acting on two separate structures.

Our results, which were based on experimental procedures which were, in some respects, less drastic than Inchley's, confirm Inchley's main conclusion that histamine constricts both inflow and outflow. These two actions tend to produce contrary effects on the volume and the observed changes in volume are, therefore, small. Under the conditions of our experiments there was no marked and constant difference between the sensitivity of the two kinds of vessels, though in some experiments the arteries appeared to be rather more sensitive than the veins. In this respect our findings differ from those of Inchley.

Acetylcholine.

Acetylcholine has been found to produce vaso-constriction in the pulmonary vessels of rabbits [Antoniazzi, 1931; Euler, 1932] and cats [Hirose, 1932]. In their experiments on dogs Daly and Euler [1932] found that acetylcholine sometimes produced vaso-constriction and sometimes vaso-dilatation. Franklin [1932] found that acetylcholine dilated the dog's pulmonary arteries and constricted the veins. We have found that cats are more sensitive to acetylcholine than dogs.

Cats. The result of a typical experiment is shown in Fig. 7. The outflow records are not included in this figure or in Fig. 8 because they showed no changes. It will be seen that small doses ($1-3\gamma$) produced vaso-dilatation, and had no definite effect on the lung volume. After a larger dose (20γ) the initial vaso-dilatation was followed by vaso-constriction and a rise in lung volume. This shows that the pulmonary veins were constricted. In another experiment, the result of which has not been reproduced and in which, when the pump was connected directly to the arterial cannula, acetylcholine (10γ) caused a rise of perfusion pressure and lung volume rather larger than that shown in Fig. 6, it was found that when the perfusion was carried out at constant pressure the same dose caused a diminution of flow and a small and indefinite fall in the volume of the lung. From these results we concluded that these doses of acetylcholine produced constriction of both inflow and outflow in the cat. When the inflow was kept practically constant by the pump, constriction of the outflow caused the rise in lung volume shown in Fig. 7, but, when the perfusion was carried out at constant pressure, the rates of inflow and outflow were both diminished and there was practically no effect on the volume. These effects were all abolished by atropine.

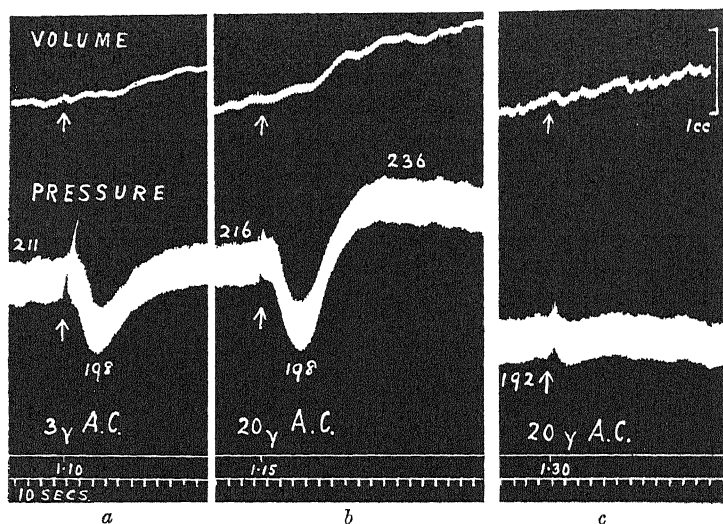


Fig. 7. Cat. Acetylcholine. Lung volume. Perfusion pressure (mm. of blood). At 1.22 l mg. of atropine sulphate was added to the blood in the venous reservoir.

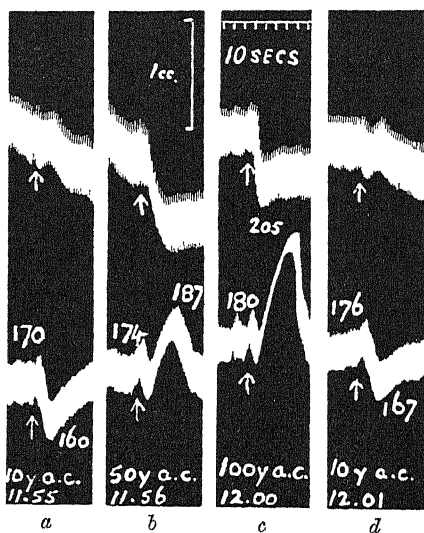


Fig. 8. Dog. Acetylcholine. Lung volume. Perfusion pressure (mm. of blood).

Dogs. In the experiment shown in Fig. 8 the effects of acetylcholine on the perfusion pressure in a dog's pulmonary artery were similar to the effects seen in experiments with cats, though the doses used were larger. A small dose (10γ , Fig. 8 *a*) caused vaso-dilatation. After larger doses the initial dilatation was followed by vaso-constriction. The dilator action of small doses was not seen in some experiments, but the constrictor action of large doses was always present. The vaso-dilatation was accompanied by a very small increase in lung volume, but, in contrast to the effects seen in cats, the vaso-constriction was always accompanied by a fall in lung volume, even when the pump was connected directly to the pulmonary artery as in the experiment shown in Fig. 8. These effects were increased by eserine and abolished by atropine. In one experiment, eserine converted a vaso-dilator effect into a vaso-constrictor one. This action was presumably due to an increase of the effective concentration of acetylcholine.

It is doubtful whether significance should be attached to the very small rise in lung volume shown in Fig. 8 *a, d* after the injection of 10γ of acetylcholine. The direction of this change would indicate that the dilator action of the drug was on the inflow. We can, however, be more confident that large doses of acetylcholine constrict the inflow and have no significant action on the outflow.

Franklin [1932] has recently published an account of the action of acetylcholine on the dog's pulmonary vessels. He found that this drug invariably relaxed isolated rings of pulmonary artery and constricted isolated rings of pulmonary vein, and deduced that it would produce a rise of lung volume. The small dilatation of the lung which followed the injection of 10γ of acetylcholine in the experiment shown in Fig. 8 *a, d* is probably the effect which Franklin predicted. It is not difficult to suggest possible explanations of the fact that he did not observe the other more marked effect of larger doses. Franklin's experiments were confined to the trunks of the vessels outside the lungs, and it is improbable that changes in the tone of these large vessels can have much influence on the blood flow. It is possible that acetylcholine has an effect on the small vessels which does not occur in the large ones. Our own failure to obtain evidence of a constrictor action of acetylcholine on the veins was probably due partly to the fact that the effects of such an action would be masked in our experiments by the increase of the inflow resistance, and partly to the fact that the acetylcholine would be mostly destroyed before it reached the veins. In any case acetylcholine was the only drug which never produced any considerable rise in the dog's lung volume in our

experiments, and we consider that it is improbable that the action of acetylcholine on the pulmonary vessels can cause the lungs to dilate appreciably in the whole dog.

Adenosine and its compounds.

Adenosine and its compounds have been shown to be present in a large number of tissues. Some of their pharmacological effects have been studied by Drury and Szent-Györgyi [1929], Bennet and Drury [1931], Zipf [1931], Ostern and Parnas [1932], Deuticke [1932] and Drury [1932]. Zipf has drawn attention to the fact that they are probably responsible for those effects of defibrinated blood which Freund [1920] attributed to the "Frühgift."

We have studied the effects on the pulmonary vessels of adenosine, muscle adenylic acid and adenylypyrophosphate. The latter substance was first isolated from voluntary muscle [Lohmann, 1931]. The effects of all these three substances in our experiments were qualitatively the same, and were in many ways similar to those of acetylcholine. The cat's vessels were much more sensitive than the dog's vessels.

In experiments with cats small doses produced vaso-dilatation, and when the dose was increased the initial dilatation was followed by vaso-constriction. The dilatation was not accompanied by any change in the volume of the lung or outflow and its site could, therefore, not be identified (Fig. 9 *a, b*). Fig. 9 *c* shows that, when the lung was perfused at approximately constant pressure, the injection of 0.25 mg. of adenylypyrophosphate caused vaso-constriction, accompanied by a fall in lung volume. This effect must have been due to an action mainly on the inflow. On the other hand, when the pump was connected directly to the artery the initial fall in lung volume was followed by a large and irreversible increase. This second effect may have been due either to prolonged constriction of the outflow or to oedema.

The effects of muscle adenylic acid and of adenosine were indistinguishable from one another when the substances were given in equimolecular doses. Both these substances were, however, much weaker in their action than adenylypyrophosphate. In Fig. 9 *a, b* it will be seen that 0.005 mg. of adenosine produced less dilator effect than a dose of adenylypyrophosphate corresponding to 0.005 mg. of its barium salt. Since the ratio of the molecular weights of these substances is 3.3, this result indicates that the addition of the extra phosphate groups to the molecule of adenosine increased its activity more than 3.3 times. In another experiment the constrictor effects of larger doses were compared,

and it was found that each molecule of adenylypyrophosphate was about equivalent in this action to fifteen molecules of adenosine. Since adenylic acid is not more active than adenosine, it is the addition of the pyrophosphate group which leads to the increase of activity. It was, however, found in one experiment that sodium pyrophosphate itself had no action

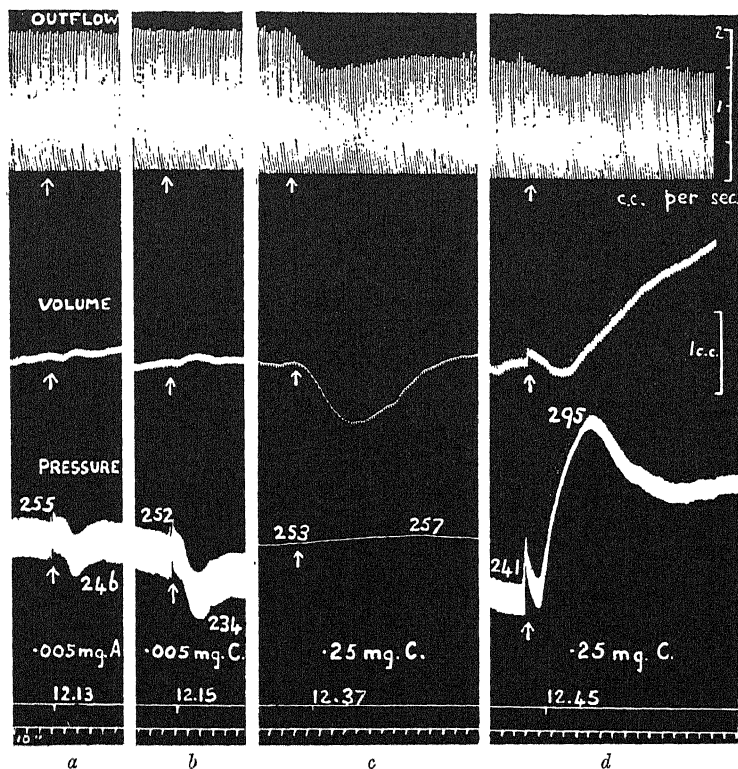


Fig. 9. Cat. A. Adenosine. C. Adenylypyrophosphate (doses in terms of weight of the barium salt). Outflow. Lung volume. Perfusion pressure (mm. of blood).

when given in comparable doses. In preparing the barium adenylypyrophosphate for injection, an excess of sodium sulphate had been added to ensure complete precipitation of the barium. A control experiment showed that quantities of sodium sulphate, considerably larger than those present in the doses used, had no action in these experiments.

The pulmonary vessels of dogs were much less sensitive, and it was not found possible to inject sufficient of these substances to compare their actions satisfactorily. In one experiment, however, it was found that a

dose corresponding to 0.5 mg. of barium adenylypyrophosphate produced a small dilatation, followed by constriction of a dog's vessels, while equivalent doses of adenosine and muscle adenylic acid had no action at all.

DISCUSSION.

The actions of the different drugs are summarized below.

Adrenaline. In dogs its chief action was constriction of the inflow. Its effect on the outflow varied. Its dilator action after ergotoxine was sometimes mostly on the inflow and sometimes on the outflow as well. In cats its chief action was dilatation of both inflow and outflow. This action was not abolished by ergotoxine. When constriction occurred, it was mostly due to an action on the inflow.

Histamine. Constricted both inflow and outflow in cats and dogs.

Acetylcholine. Small doses produced dilatation in cats and sometimes also in dogs. Larger doses produced constriction. In cats this occurred both on the inflow and outflow, but in dogs the action was mostly on the inflow.

Adenosine compounds. Small doses produced dilatation and larger doses constriction. In cats the constrictor effects of large doses were mostly due to an action on the inflow. Adenylypyrophosphate produced both effects much more actively than either adenosine or muscle adenylic acid.

Though we have recorded changes in the volume of blood in the lungs, our discussion has hitherto been confined to the consideration of the contribution of the different parts of the vascular system to the total resistance to the flow of blood. Most of the effects observed have been mainly due to vaso-motor changes in the inflow, but evidence has been presented which indicates that the action of histamine and adrenaline (see Figs. 2-6), and possibly also acetylcholine and adenosine, on the tone of the pulmonary veins may have an appreciable effect on the total resistance of the lungs to the flow of blood.

We must now consider what direct significance our results may have in regard to the effect of the drugs we have used on the blood content of the lungs in the intact animal. This is of importance from two points of view. In the first place, the lungs form one of the most important potential reservoirs for blood in the body [Magnus, 1930]. In the second place, the blood content of the capillaries is one of the factors which influence the length of time during which blood is exposed to the air in the alveoli. One of the factors controlling the blood content of the lungs is the hydro-

static pressure in their capillaries. This pressure must always be less than that in the pulmonary artery and greater than that in the left auricle. The mean of these two pressures is sometimes taken as an estimate of the capillary pressure [Magnus, 1930]. It is possible, however, that the stimulation of nerves or the application of drugs might produce changes in the capillary pressure, by acting selectively either on the arteries or the veins, so that the capillary pressure approached either that in the pulmonary artery or to that in the left auricle. The changes which we have recorded in the lung volume have been attributed to such a selective action, but these changes in volume were very small. It is just possible that vaso-motor actions might produce much larger changes under physiological conditions when the lungs are being normally ventilated, but it is improbable that this action is of much importance. The result shown in Fig. 3 may be taken as a demonstration that a drug may have an action on the outflow contrary to its action on the inflow, and that such an action can be detected by the methods we have used. This result was of doubtful interpretation, exceptional in nature and inconstant in appearance. In every other case in which an action on the pulmonary veins has been demonstrated or suspected this action has been in the same direction as the action which the drug had on the arteries in the same experiment. The pulmonary veins have, in fact, responded in such a way as to neutralize the changes in lung volume which would have occurred if the action of the drug had been solely on the inflow. There is thus no reason for supposing that the pulmonary veins play a part in the control of the blood content of the pulmonary circulation which is in any way comparable with the part which the hepatic veins have been found to play in the control of the blood content of the portal circulation.

SUMMARY.

1. The lungs of dogs and cats were perfused with blood in such a way that either the perfusion pressure or the rate of flow could be maintained approximately constant. The blood content of the lungs was recorded by means of a plethysmograph.
2. The details of the effects seen are summarized above at the beginning of the discussion.
3. The plethysmographic record indicated that the most striking effects of adrenaline, histamine, acetylcholine and adenosine compounds were mainly due to an action on the pulmonary arteries, but evidence was obtained that changes in the tone of the pulmonary veins might also

have an appreciable effect on the total resistance of the pulmonary vessels to the flow of blood.

4. In almost every case where definite evidence was obtained that a drug was acting on the pulmonary veins, this action was such as to neutralize the change in lung volume which would have occurred if the drug had acted solely on the inflow.

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STUDIES ON THE PHYSIOLOGY OF REPRODUCTION.

V. The adrenal cortex in pregnancy and lactation.

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OUR finding, in recent experimental work [Andersen and Kennedy, 1932], of a significant difference between the adrenal weight of the rat at œstrus and at diœstrus has led us to investigate the adrenal changes in pregnancy. Without a preliminary knowledge of the changes in the non-pregnant animal it is impossible to have carefully standardized controls for the pregnant ones, and such knowledge has not been available to former workers. It was hoped that a series of pregnant rats of the same strain, kept on the same diet and in the same environment as that used in our former experiment, might yield valuable information.

Most recent text-books and reviews refer to an hypertrophy of the adrenals during pregnancy as an accepted fact. A careful analysis of the literature on the subject reveals that this belief is based on a rather small amount of data, and that the controls used in most of the experiments were inadequate, not only because the œstrus changes were unknown and disregarded, but because the possibility of an excessive hypertrophy of the cortex in pregnant animals that are infected over the non-pregnant ones as suggested by Donaldson [1924] in rats was not considered. Moreover, the changes described in the literature vary not only in different species, but in the same species as studied by different people. It is possible that not all mammalian adrenals respond similarly to pregnancy. The work on each species will therefore be considered separately.

Most of the work has been done on rodents, especially on guinea-pigs. The first report was by Guieyette [1899, 1901], who gave a lucid description of various changes in the adrenals of his pregnant animals, the chief of which were the following: the cells of the middle third of the cortex were increased in size and contained larger vacuoles; the cells of the inner portion of the fascicularis contained a greater number of granules staining with iron hæmatoxylin; and those of the reticularis

contained a greater number of these siderophil granules as well as an increase in pigment granules. He found no mitoses and concluded that the increase was in the size, not in the number of cells. The zona glomerulosa was unchanged. The animals were killed between the tenth and fifty-second day of pregnancy. The chief criticisms of his work are that his controls were males, except for one immature female 2 months old, that no records of the number and ages of animals were given, and that no note was made as to infections in his animals. In view of the large size of the female adrenal as compared with the male, the first of these objections goes far to invalidate his findings as the specific effect of pregnancy. Marrassini [1906] reported on ten pregnant guinea-pigs with nine non-pregnant female and fourteen male controls, and described an increase of lipoids in the fascicularis but no change in the other zones. He found a greater variation in the weight of the adrenals in both non-pregnant and pregnant females than in the males. The three animals killed in early pregnancy resembled the non-pregnant females, while the three in late pregnancy and the four killed post-partum had larger adrenals. Inspection of his figures reveals the fact that six of the female non-pregnant animals were much lighter in weight than the pregnant ones. The remaining four are in the same weight range as the pregnant ones, if the probable weight of the uterine contents be considered, and the adrenals in these animals are in the same range of weight as the pregnant ones, rather less, if relative weight be considered. The male adrenals are smaller than those of the non-pregnant females according to his figures, although he describes them as being about the same. Ciulla [1910] also confirmed Guieyesse, but mentioned no controls and gave but few details. He also studied the proteolytic enzymes of cortical tissue and found about the same quantity in pregnant and non-pregnant animals, with wide variations. He quotes Costa [1906] as reporting a decrease in the lipolytic and proteolytic activity of the adrenal cortex during pregnancy.

Kolmer [1912] examined three pregnant, six lactating, and three non-pregnant guinea-pigs and confirmed the findings of Guieyesse with the addition of many mitoses at about the time of parturition. Kolde [1913] also confirmed the findings of Guieyesse in his study of four pregnant and two non-pregnant animals, one of the latter being very old. These observations are based on so few animals, in view of the range of variation of adrenal weight, that they may be disregarded.

Sternberg [1915] had a series of thirteen pregnant, eight post-partum, and five non-pregnant control guinea-pigs and confirmed Guieyesse as to hypertrophy of the fascicularis with increased lipoid

and without mitoses. He also described an hypertrophy of the glomerular zone. He includes a table of data which reveals the fact that actual weights are reported on only three of the five controls and that these three are small animals. Calculation of the weight of adrenal per kg. of body weight based on his data gives a result in the controls within the same range as in the pregnant animals. Sternberg's work can therefore be disregarded.

Verdozzi [1914, 1917] presented a series of twenty-four pregnant and twenty post-partum animals, and a description of the adrenal in the non-pregnant controls, the number of which was not given. He calculated the relative weight and made from his data a chart, in which there is a high peak at the end of the first half of gestation produced by the figures from two animals. The remaining figures for pregnant animals are low on the average but vary greatly. In eleven post-partum animals the young were left with the mother, and in nine they were removed. The figures for the former series are definitely higher than for the latter and appear significant.

The most recent work on guinea-pigs is that by Castaldi [1922] on a series of eleven pregnant animals with a number of non-pregnant controls, of various ages, of which six were older than 3 months. He confirmed Guieyette. A study of the figures which he presents shows so great a variation that the difference is not significant, especially if the relative weight is calculated.

Other rodents that have been studied are the rat, mouse and rabbit. Herring [1920] found that the mean relative adrenal weight in nine pregnant rats was 0.22 g./kg. and in nine non-pregnant controls it was 0.20 g./kg., a difference that is not significant in view of the variation and the number of animals. Donaldson, in twenty-nine pregnant and lactating rats, with a mean body weight of 167 g., found a mean relative adrenal weight of 45.3 mg., while in twenty-nine non-pregnant controls with a mean body weight of 163 g. the mean adrenal weight was 44.7 mg., or practically the same. He pointed out a most interesting fact, that the infected pregnant animals had a much higher adrenal weight than the infected non-pregnant ones. Forty-seven pathological controls with a mean body weight of 174 g. had a mean adrenal weight of 47.9 mg., while fifty-one pathological pregnant and lactating rats with a mean body weight of 163 g. had a mean adrenal weight of 57.8 mg. In a later study Donaldson [1928] showed that the relative volumes of the medulla and cortex were the same in pregnant and non-pregnant animals.

In mice the problem is complicated by the presence, in this species, of

the X-zone lying between the fascicularis and medulla. Three workers, with an adequate number of animals, have described a decrease in size of the adrenals and the rapid disappearance of this zone during pregnancy [Tamura, 1926; Howard-Miller, 1927; Deanesly, 1928]; Howard-Miller [1927] and Deanesly [1928] describe this phenomenon as the hastening of the normal age involution of this zone. All three workers investigated the presence of œstrus changes: adrenal hypertrophy was described by Masui and Tamura [1926], but was not found by Howard-Miller [1927] and Deanesly [1928].

Gottschau [1883] made the first observations on rabbits. In a series of twenty pregnant rabbits and nineteen non-pregnant females, he observed a slight decrease in size in the adrenals of the pregnant animals, while the adrenals of seven males were still smaller. Elliott and Tuckett [1906] give low figures in two pregnant and three non-pregnant females. Ciulla analysed the cortex of rabbits for fat and lecithine and found that the fat content was 10 p.c. by weight in the non-pregnant and 18 p.c. in the pregnant animals. Lecithine formed 50 p.c. of this in the non-pregnant and 72 p.c. in the pregnant. He gave but few details, and did not mention the number of animals, the age and weight, or the sex of the controls. Kolde [1913] found no difference between five pregnant and eleven non-pregnant females. Sternberg [1915], who studied eight pregnant and two lactating rabbits and five non-pregnant controls, reported changes similar to those in guinea-pigs. He gives complete data on only one control, but states that there is hypertrophy in the pregnant animals. This finding, in the face of the previous work and his inadequate controls, leaves one sceptical of his work, not only on rabbits, but also on guinea-pigs.

No observations on dogs have been found, but the figures given by Elliott and Tuckett on two pregnant and two lactating cats show about the same relative weight of the adrenals as in the non-pregnant females of his series.

The diagram given by Kolmer [1918], based on observations of eight pregnant, one lactating, sixteen non-pregnant female and twenty-five male moles, indicates adrenal enlargement during the breeding season (February to April) in both sexes. This enlargement was found in both pregnant and non-pregnant animals during the season of rut. The factor of seasonal variation in a hibernating animal complicated the picture.

In man, the most considerable series described in detail is that of Sambalino [1910], who reported a series of twenty-six cases of women dying in pregnancy or the puerperium. Many of these died after an

abortion or immediately post-partum of various causes. An abstract of his case histories reveals the following facts: he described marked hypertrophy in three cases, one of which died of pyelonephritis, and the others of puerperal infection; slight to moderate hypertrophy in sixteen cases, the cause of death being hæmorrhages in three, eclampsia in three, chronic nephritis in four, post-partum infection in two, pulmonary tuberculosis in one, pneumonia in two and pulmonary thrombosis following femoral thrombosis in one; adrenals of normal size in four cases, dying of acute nephritis, eclampsia, abdominal sarcoma and puerperal infection respectively; and very small adrenals in three cases dying of nephritis (two cases) and pernicious anæmia. He concluded that increased vacuolization in the zona fascicularis and increased pigment in the zona reticularis were changes due to pregnancy, and that adenomata were common in the cases of eclampsia and nephritis. However, according to the descriptions of individual cases, these are not always present. Moreover, the size of the adrenal was estimated from its appearance in the microscopic sections. There is no correlation between the degree of hypertrophy and the age of the patient or the number of pregnancies. The constant presence of any one change was not demonstrated. Chirié [1908] reported a series of twenty-eight autopsies on women dying during the terminal portion of pregnancy or soon after parturition. Of these, twelve died of eclampsia, four of nephritis, and the remainder died of various causes, chiefly infection. He observed hypertrophy of the cortex in most of the cases of eclampsia and nephritis with hypertrophy of the medulla in many of these cases. Hypertrophy of the cortex was found twice and hypertrophy of the medulla was not present in the remainder of the series. Cortical adenomata were about equally frequent in all types of cases. He concluded that the hypertrophy was a response to the intoxication resulting from renal changes. Minervini [1904] reported a case of a woman who died in the seventh month of pregnancy from eclampsia, and Gaifami [1907] reported four cases, in two of which the cause of death was streptococcus infection, and in the other two shock from hæmorrhage. Both observers described increased lipoid in the fascicularis and increased pigment in the reticularis. Kolde [1913] described the adrenals of five pregnant and five non-pregnant women. He found the zona fascicularis and zona reticularis large, and the glomerulosa small and with mitoses. There were no adenomata in his case of eclampsia.

Landau [1915], aware of Sternberg's work in the same laboratory, in a careful study of three hundred autopsies of adults of both sexes, was surprised to find no difference between adrenals of the cases dying in

pregnancy or during the puerperium (number of cases not given) and the remaining non-pregnant ones. Goormaghtigh [1922] added one case in which he found hypertrophy of the fascicularis with an increase of lipoid, and atrophy of the reticularis. The difficulties of evaluating autopsy material are manifest, especially in view of Donaldson's observations in rats of marked adrenal hypertrophy in infected animals, but not in uninfected ones, and Goormaghtigh's observations on the variability of the changes in infections following war wounds.

A critical review thus leads us to the conclusion that the evidence is against hypertrophy of the adrenal during pregnancy in the rat, mouse, rabbit and the cat. The hypertrophy found during the breeding season in the mole is found in both pregnant and non-pregnant animals as well as in males. There remain the conflicting findings of various observers of the guinea-pig. It has been shown that none of the work on the adrenals of pregnant guinea-pigs has been adequately controlled. It is possible that this animal differs in its reactions from other rodents, but the question must be left open until careful work has been done with non-pregnant female controls of the same breed, age and weight, with a study of possible changes associated with the oestrus cycle and with scrupulous elimination of infected animals. Notwithstanding the limitations of work on post-mortem material, the weight of evidence is against hypertrophy of the adrenals in pregnant women.

TECHNIQUE.

The animals used were of the same strain and kept on the same diet and under the same conditions as those used in our studies on the adrenal in oestrus [Andersen and Kennedy, 1932]. They were all bred in our laboratory, so that the exact age was known. The majority were killed during their first pregnancy, although one of the uninfected and several of the infected ones had previously had litters. All but five were killed between the ages of 120 and 200 days, the remainder being a few weeks older. The cycles were followed by means of vaginal smears, and the animals were mated for one night during oestrus, so that the exact length of pregnancy was known. They were then killed in groups of four to six at the following periods of pregnancy and lactation: the tenth, eighteenth and twenty-first days of pregnancy; during parturition, with part of the litter born and part unborn; and on the first, seventh, fourteenth and twenty-first days of lactation. In all, the series comprises twenty pregnant and twenty-one lactating rats. Forty-one more animals

were used, but these were animals having some infection of the lungs or ears and were made to form a separate series. The experiments were carried on over the course of about 18 months as animals were available, and no seasonal variations were noted.

The animals were killed with chloroform, and the body weight both with and without the products of conception was noted. The animals were examined for infections, especially of the lungs and middle ears. The pituitary, thyroid, adrenals and thymus were then weighed in a closed weighing bottle and fixed in Zenker's solution. In some cases from each stage one adrenal was fixed in formalin. Both adrenals from eight animals were serially sectioned and stained with hæmatoxylin and eosin: of these, three were from animals killed on the eighteenth day of pregnancy, two on the first day post-partum, and three from the twenty-first day of lactation. Frozen sections stained for fat with Scharlach R were made of adrenals from animals of each phase. The series of animals studied in regard to œstrus and diœstrus and previously reported [Andersen and Kennedy, 1932] were used as controls.

The weights of the organs are calculated in terms of g. per kg. of total body weight and of net body weight. The total body weight is considered as the weight of the mother plus the uterus and its contents in the pregnant animals, or plus the weight of the litter in the lactating ones, conceiving of the young either unweaned or unborn as dependent on the mother for nourishment. The net body weight is the weight of the mother after the uterus and contents have been removed, or the weight of the lactating mother without regard to the litter. The results in regard to the adrenals will be reported here, and those in regard to the pituitary and thyroid will be reported in a subsequent paper.

In addition a group of four pseudo-pregnant rats, killed 1 week after mating with a vasectomized male, were included. Four rats killed after an œstrus period of 3 weeks or more, and nine non-pregnant animals dying of spontaneous infections were also studied for comparison.

No animals were used which were known to be infected at the time of mating, and the high percentage of infections is surprising in view of this limitation, the adequate diet, ample light and cage space, and careful attention to cleanliness. All the infected rats had the same type of lung infection so commonly found in rats, in which smaller or larger chronic abscesses are scattered through the lung, with occasional solidification of one or more lobes. A few also had middle ear infections. A few of the animals in the infected series were about a year old, and were among the first to be examined. Unsuspected infections were found much more

commonly after the age of 6 months, and for the remainder of the experiment only young animals between 90 and 150 days of age at the time of mating were used. Since the degree of infection cannot be easily measured, and some rats having only a few abscesses had large adrenals, all animals having any evidence of infection whatsoever were eliminated from the normal series.

The calculation of the probable error was made from the formula

$$\text{P.E.} = \frac{2}{3} \sqrt{\frac{\sum x^2}{N}}.$$

DATA.

The mean relative adrenal weight at various stages of pregnancy is given in Table I. It may be seen that in normal animals the relative adrenal

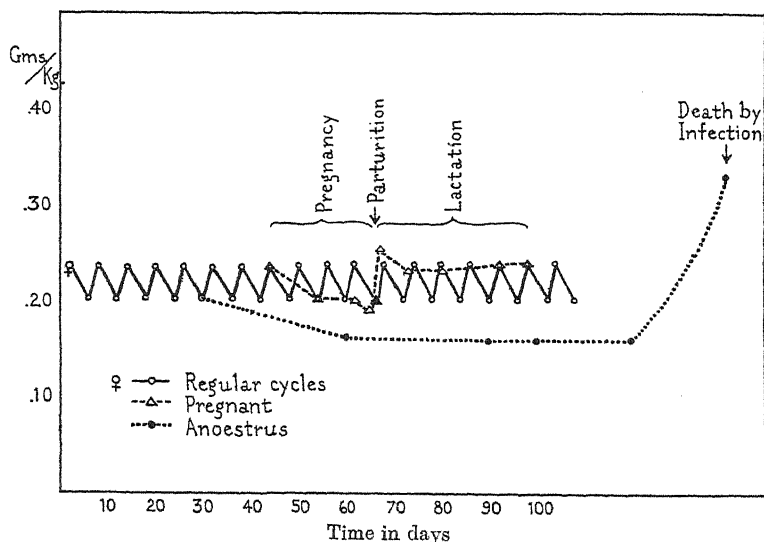


Fig. 1. The relative weight of the adrenal gland in pregnant, lactating, non-pregnant and anoestrus female rats. Body weight approximately 200 g.

weight is about the same in various stages of pregnancy. It is also quite uniform throughout lactation and is slightly heavier than in pregnancy. The largest figure, at 1 day post-partum, is due to a single large reading of 0.332 g./kg. in a rat in which no infection was discovered. This is more than 20 p.c. larger than any other rat of the entire series of normal pregnant or lactating animals, and is in the range of weight found at oestrus. In some cases the rat has a single oestrus period immediately

TABLE I. The mean weight of the adrenal glands in various stages of pregnancy.

Uninfected animals						
Stage	No.	Body weight in g.		mg.	Adrenals	
		Total with young	Net without young		g./kg. total body weight	g./kg. net body weight
10 days pregnant	4	204	203	41.6	0.205	0.205
18 " "	7	234	208	41.2	0.177	0.200
21 " "	4	255	199	37.9	0.150	0.192
Parturition	4	247	195	42.0	0.171	0.217
1 day post-partum	5	243	197	48.1	0.201	0.248
7 days post-partum	5	253	197	45.2	0.179	0.230
14 " "	5	351	206	47.7	0.138	0.234
21 " "	6	450	223	48.1	0.108	0.217

Infected animals						
Stage	No.	Body weight in g.		mg.	Adrenals	
		Total with young	Net without young		g./kg. total body weight	g./kg. net body weight
10 days pregnant	5	210	209	47.3	0.227	0.227
18 " "	10	224	201	51.6	0.233	0.262
21 " "	6	274	230	54.6	0.200	0.239
Parturition	—	—	—	—	—	—
1 day post-partum	11	259	213	53.2	0.208	0.253
7 days post-partum	—	—	—	—	—	—
14 " "	—	—	—	—	—	—
21 " "	9	373	211	48.2	0.129	0.229

post-partum and it was felt that this might be the case here, but unfortunately no vaginal smear was taken and this point could not be ascertained at the time that the large weight was discovered. The two animals of the same stage which were killed subsequently were carefully examined and had no oestrus changes in the uterus or in the vaginal smear, and, at the same time, had adrenals which corresponded in weight with those of the remaining rats of the series. The point, therefore, remains obscure.

The mean weights for the series of pregnant and for the series of non-pregnant animals are given in Table II, with the figures for non-pregnant oestrus, dioestrus, anoestrus animals of approximately the same weight for controls. A group of rats dying of spontaneous lung infections is added for comparison with the less severely infected pregnant and lactating animals. The weights for the pregnant animals correspond with those of the dioestrus series and are much less than those of oestrus. The weights for the lactating animals are higher, with a probable error that

TABLE II. The mean absolute and relative weight of the adrenal glands in pregnant and lactating rats as compared with non-pregnant female rats of various types.

Uninfected animals				
Stage	No.	Net body weight g.	Adrenals	
			Actual weight mg.	g./kg. net body weight
Pregnancy	15	204 ± 18	40.4 ± 3.4	0.199 ± 0.010
Parturition	4	195 ± 11	42.0 ± 0.7	0.217 ± 0.013
Lactation	21	206 ± 18	47.3 ± 3.8	0.232 ± 0.021
Pseudo-pregnancy	4	203 ± 5	45.8 ± 3.3	0.227 ± 0.021
Œstrus	13	Over 180	49.2 ± 4.3	0.237 ± 0.016
Dicæstrus	6	"	42.0 ± 2.4	0.212 ± 0.006
Anæstrus	4	177 ± 7	29.0 ± 3.4	0.164 ± 0.018
Rats dying of infection	—	—	—	—

Infected animals				
Stage	No.	Net body weight g.	Adrenals	
			Actual weight mg.	g./kg. net body weight
Pregnancy	21	211 ± 19	51.5 ± 6.1	0.247 ± 0.033
Parturition	—	—	—	—
Lactation	20	212 ± 22	50.9 ± 5.4	0.243 ± 0.024
Pseudo-pregnancy	—	—	—	—
Œstrus	—	—	—	—
Dicæstrus	—	—	—	—
Anæstrus	—	—	—	—
Rats dying of infection	9	160 ± 26	69.0 ± 7.6	0.442 ± 0.046

is fairly significant. There is no correlation between the size or total weight of the litter and the size of the adrenal. The adrenal weights for the pseudo-pregnant animals correspond with those of the pregnant ones, as might be expected. The somewhat higher mean is the result of one large figure, while the other three correspond closely with the pregnant ones. It is obvious that hypertrophy of the adrenals in relation to reproductive phenomena in the rat is found in connection with œstrus and to some extent with lactation, but not with pregnancy.

The infected animals show much greater variation in the adrenal weight than the uninfected ones, with some increase in mean relative weight. The figures for the various phases of pregnancy and lactation cannot be interpreted to mean a greater or lesser adrenal sensitivity to infection, because the degree of infection varies and in some groups there are a greater number of severely infected animals. There is a rough correlation between the degree of infection and the degree of adrenal hypertrophy and only a few of the slightly infected animals have larger adrenals

than the normal ones. For example, of the five infected rats killed on the tenth day of pregnancy only one was severely infected, with consolidation of one lobe of the lung and many small abscesses in the other lobes, while the remaining four rats had only a few scattered small abscesses. The severely infected rat had an adrenal weighing 0.281 g./kg., while the heaviest of the others weighed 0.228 g./kg. Six of the eleven animals killed on the first day post-partum had quite severe infections, and most of these had large adrenals. The mean weight for this stage is consequently large. The rule does not hold exactly, however, for several of the severely infected animals in the series had small adrenals, and another factor or other factors must be present.

Hypertrophy of the adrenal in these spontaneous lung infections in the rat occurs also in non-pregnant animals. In a series of nine non-pregnant animals dying of such infections the mean actual adrenal weight was 69.0 mg. and the mean relative adrenal weight was 0.440 g./kg., a figure very much higher than that for the animals with milder infections.

A study of serial sections of the pregnant animals reveals no striking changes from the appearance of the diestrus rat which has been previously described. There is a little more lipoid in the fascicularis in some of the lactating animals, as shown by the Scharlach R stain on frozen sections, but this is not so striking as to enable one to distinguish between the pregnant, lactating and diestrus adrenals with any degree of consistency.

DISCUSSION.

It is obvious from the data presented here that, in the normal rat, there is no adrenal hypertrophy during pregnancy. The weight and histological appearance correspond with that of non-pregnant diestrus animals. This does not necessarily hold true for other species, such as the guinea-pig, in which the œstrus cycle is longer. In our series of four rats which had had no œstrus cycles for some weeks, the adrenals were smaller than those of either the diestrus or pregnant animals. In the short cycle of the rat the post-œstrus or uterine phase of the cycle is so closely followed by the next œstrus that it is difficult to surely distinguish the post-œstrus from the diestrus phase. It is quite possible that in an animal having a longer cycle, such as the guinea-pig, the adrenals continue to decrease in size after the post-œstrus period, assuming that œstrus hypertrophy in other animals occurs, which is as yet unproven.

An interesting point is brought out by a comparison of the actual weights and the relative weights in relation to the total and net body

weights as described above. In a study of the figures for the individual rats it is seen that the most constant figure is that of the relative weight per net body weight. The actual weights vary with the body weights. This greater uniformity and, therefore, greater significance of the relative weight per net body weight over the actual weight is also shown by the probable error, which is about 5.0 p.c. of the mean in the case of the relative weight and 8.6 p.c. of the mean in the case of the actual weight in pregnant animals. The relative weight in relation to the total body weight decreases as the products of conception increase, but varies greatly (Table I). For example, in one rat weighing 196 g. and 54 g. of uterus and contents, the actual adrenal weight is 39.5 mg., the relative weight per total body weight is therefore 0.158 g./kg., while the relative adrenal weight per net weight is 0.202 g./kg. or very near the mean for dioestrus and pregnancy. This means that the physiological burden on the adrenal is not sufficiently increased or altered by oestrus, lactation and infection. Whatever may be the function of the adrenal, it does not appear to be involved in the preparation of nutrient material for the embryos. The embryos themselves acquire relatively large adrenals at a very early stage.

That the adrenals of pregnant rats hypertrophy more readily in the presence of infection than those of non-pregnant animals as suggested by Donaldson [1924] is not clear, since the majority of the non-pregnant female rats of our colony with even a mild degree of infection have long periods of anoestrus. Those with slight infections have the very small adrenals found in the uninfected anoestrus rats, while those with more extensive processes have adrenals of varying sizes up to 0.090 g. A series of non-pregnant infected animals would, therefore, include adrenals both smaller than and as large as those in a series of pregnant infected rats, and the comparison is difficult. In our relatively short series the greater frequency of severe infections during lactation over the number found in the earlier stages of pregnancy may not be significant, but it is suggestive.

The question of a possible relationship between the corpus luteum and the adrenal cortex has interested many investigators from Mulon [1906] and Watrin [1914] on. In recent years several papers have appeared which deal with a difference in the survival period of pregnant and non-pregnant animals following adrenalectomy. A prolongation of the survival period in pregnant animals was first described by Stewart [1913] in cats; but the survival period in most of his animals was shorter than is considered usual in non-pregnant animals at the present time. A more marked prolongation of the survival period was reported by

Rogoff and Stewart [1927] in a series of seventeen pregnant dogs. The maximum survival period in seventy-four non-pregnant and male animals was 15 days (two dogs), while the majority died between the fourth and twelfth day. Of the seventeen pregnant animals five lived for 13 to 15 days and eight lived over 17 days, some of the latter surviving many weeks. They believed that this prolonged survival period was due to the presence of the corpus luteum, since they also obtained unusually long survival in two non-pregnant animals which were adrenalectomized towards the end of an œstrus period and therefore had corpora lutea. Corey [1928] repeated the experiment on cats and did not find a prolongation of the survival period in twelve pregnant animals as compared with three non-pregnant females and three males. The matter must therefore be left open, although the former report is more convincing, both because of the experience of the workers, and because of the greater significance of positive over negative findings in a question where operative technique is so important. The report by Carr [1931] of a prolonged survival period in pregnant rats may be disregarded, since non-pregnant rats may survive adrenalectomy indefinitely, presumably because of the presence of accessory fragments of cortex. If we accept the findings of Rogoff and Stewart, their interpretation must be left until further elucidation of the cortical function; the two hypotheses that appear possible are that the corpus luteum can act as a substitute for the adrenal cortex—a theory based largely on histological similarities; or that, for some other reason, less of the secretion of the adrenal cortex is necessary during pregnancy.

The present confirmation of Verdozzi's findings of hypertrophy of the adrenal during lactation does not greatly clarify the question of the relation between the corpus luteum and the adrenal. A large adrenal is found during œstrus when the corpora lutea are not active and during lactation when they are; a small adrenal is found during pregnancy and pseudo-pregnancy when the corpora lutea are active, and during diœstrus when they are not; and a still smaller one during anœstrus when neither corpora nor follicles are active. Yet the pregnant dogs of Rogoff and Stewart survived adrenalectomy longer than the non-pregnant ones. The resolution of these apparently incompatible phenomena into a logical hypothesis cannot be accomplished as yet, but it would seem probable that the relationship between the corpus luteum and the adrenal cortex is not a direct one.

It has not yet been proven that a hypertrophied adrenal is an over-active one, and this will doubtless not be settled until the function of

the cortex is known, since the hypertrophy of the adrenal is usually due to hypertrophy of the cortex. The conditions in which hypertrophy has been found are beriberi, starvation [McCarrison, 1921], some infections, œstrus or the mating season, and lactation; these are all occasions of especial strain on the organism. However, other types of strain are not accompanied by adrenal hypertrophy, and the common denominator in this series is not obvious.

CONCLUSIONS.

1. The mean actual weight of the adrenals in a series of fifteen pregnant rats is 40.4 ± 3.4 mg. The mean weight as expressed in terms of g. per kg. of the body weight of the mother minus the products of conception is 0.198 ± 0.010 . There is but a small variation in the relative weights so calculated.

2. The corresponding figures for a group of four rats killed during parturition are 42.0 ± 0.7 mg. and 0.217 ± 0.013 g./kg. There is no definite change either just before, during, or just after parturition.

3. The mean actual weight of the adrenals in a series of twenty-one lactating rats is 47.3 ± 3.8 mg. The mean relative weight, calculated as described above, is 0.232 ± 0.021 g./kg. This is higher than in the pregnant animal, and appears to be a significant increase.

4. As compared with the non-pregnant controls described in a previous paper it is found that the relative and absolute weight of the adrenal in pregnant rats is the same as in non-pregnant rats examined during diœstrus, and is considerably less than the weight during œstrus. It is greater than the weight in animals killed after a long period of anœstrus. The adrenal weight in lactating animals is slightly less than that during œstrus.

5. A series of four pseudo-pregnant rats had adrenals resembling those of pregnant animals.

6. In pregnant and lactating rats which are found at autopsy to have lung infections the adrenal weight is much more variable than in normal animals. In moderate or severe infections it is 25–50 p.c. greater, and in the slight infections it is sometimes greater and sometimes not. The degree of enlargement corresponds roughly to the degree of infection.

7. The histological appearance of the adrenal in the pregnant animal resembles that in the non-pregnant diœstrus one. The adrenal of the lactating animal contains a little more lipoid in the fascicularis.

8. A critical survey of the literature reveals that the balance of evidence is against hypertrophy of the adrenals during pregnancy in the

rat, mouse, rabbit and cat. There is hypertrophy of the adrenals of both sexes during the season of rut in the mole, but this has not been shown to be characteristic of pregnant animals in particular. The evidence for such hypertrophy in the guinea-pig is not adequately controlled in any series and the truth of the matter is in doubt. The studies on human autopsy material yield conflicting results, although the most reliable work is against such hypertrophy.

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THE DIFFERENTIAL PHARMACOLOGY OF THE INFERIOR VENA CAVA OF SOME MAMMALS.

By K. J. FRANKLIN.

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IN rabbits which have been killed by a blow on the head and have then been bled from the jugular veins, and in rabbits and cats killed and bled simultaneously by the humane killer, I have frequently found the inferior vena cava contracted caudal to the renal vein entries and apparently widely dilated between these and the liver. In anæsthetized animals, also, I have found that it is not always easy to insert a wide cannula into the posterior part of the vein because of its great contractility. I have, for instance, seen it shrink after being cut from a diameter of approximately 5 mm. to the thickness of a thread.

The reason for the contraction in cases of hæmorrhage may be, as suggested by the papers of Rous and Gilding [1929] and of Gilding [1929], that the blood supply to the muscles and skin is temporarily reduced while that to the upper part of the intestine, whence fresh fluid would naturally enter the body, is kept normal and, with it, that part of the inferior vena cava into which the portal vein empties. In cases of small hæmorrhage it is probable [Jarisch and Ludwig, 1927] that the vena cava would not be concerned, but only the portal system. That the extra contractility of the caudal portion of the vein may have another function besides this emergency one, however, is suggested by the normal lie of the venæ cavæ. This has already been pictured in the case of the rabbit and of the cat [Franklin, 1932 *a*], and the lie in the dog, sheep, and guinea-pig is similar in that, in the standing posture of the animal, the inferior vena cava ascends from the iliac veins to the renal veins and descends from these latter to the heart. The flow in the first part is against gravity, in the second part with it, and there is a tendency for veins which have to contend against gravity to be better equipped, *ceteris paribus*, with smooth muscle. There is also the point that the suprarenal veins discharge into the part of the vena cava between the renal veins and the liver, and it would be awkward for the body's economy if this portion responded more than the distal one to the secretion of the suprarenal medulla.

On all these counts it seemed worth while to compare the pharmacological reactions of the iliac portion (between the iliac and renal veins) and of the renal portion (between the renal veins and the liver) of the inferior vena cava. Waterman [1930] has shown that the vein¹ contracts with parasympathomimetic drugs, and I have also found contractions of minor degree with such reagents. To eliminate the possibility of parasympathetic stimuli being the cause of the contraction *in situ*, I took a ring which was so contracted and added atropine sulphate to the Ringer's solution in the beaker. There was no relaxation, so it seemed probable that the contraction was chiefly muscular or sympathetic in origin, and the latter interpretation is supported by the experimental results.

Veins from three species, namely, the rabbit, the cat and the dog, have been used, and have been tested by two techniques *in vitro*, and also by injection of adrenaline *in situ*.

In the experiments *in vitro* the reactions of vein rings to adrenaline 1:100,000 have been recorded. This concentration of the drug has been employed as likely [Franklin, 1932 *b*] to give a maximal effect, a conclusion justified by the fact that subsequent additions of 1:200 barium chloride usually caused no further contraction or, at best, only a small one. The recording was done by means of an optical recording apparatus, already described apart from small modifications [Franklin, 1930], and the movements were magnified 150 to 400 times, according to the size of the vein ring used.

Two different techniques were employed, as stated above. The first depended on the fact that the vein has a maximum distension point. This has already been pointed out in the case of the cat [Franklin, 1931], and was found to obtain likewise in the rabbit and in the dog (*q.v. infra*). This being so, it was assumed that, if the counterpoise in the case of any particular vein ring were so adjusted that it was the minimum weight which would give a maximum length of the vein between the hooks, this length corresponded to the maximum distension of the vein *in situ* and could be used as a zero. The only drawbacks to this were, first, that the measurements had to be made at room temperature, and on immersion in oxygenated Ringer's solution at body temperature there was a certain amount of contraction; and secondly, that this heat contraction affects also the connective tissue elements. If such a ring, however, is left for some time, it relaxes again, and one is probably not far wrong in taking the room temperature length as zero; at all events, the qualitative ratios

¹ Although not stated in the original paper, the part of the vein Waterman used was the abdominal, and not the thoracic.

are not affected, as comparison with the results of the second technique shows. The zero was recorded as soon as the vein ring was immersed in the Ringer's solution, and the difference between the zero and the highest reading produced by subsequent addition of adrenaline 1 : 100,000 was regarded as the maximum effect of the adrenaline for comparative purposes.

The second technique was suggested by Prof. J. A. Gunn, and depended on the observed fact that smooth muscle tends to relax right down if left for a long time in oxygenated Ringer's solution at body temperature. The length to which vein rings had relaxed after an hour in such an environment was, in these experiments, taken as zero, and the contraction thereafter produced by adrenaline 1 : 100,000 was recorded. At the maximum height of adrenaline contraction the lever holding the vein was fixed by tightening the side screw of the mirror spindle, and the length between the hooks was measured. The original length at zero was calculated from this, and thereafter the percentage diminution in circumference. The ratios between the contractions of the iliac and renal portions recorded by this technique agreed closely enough with the ratios found by the first technique on neighbouring vein rings. For instance, in the case of one dog's vein, they were respectively 2.5 and 2.2 : 1. The actual percentage contraction measured by the first technique was less than that measured by the second technique, but this was to be expected in view of the greater counterpoise used in the first method.

The experiments showed that the iliac portion of the vena cava of the cat, rabbit and dog was always more reactive to the given dose of adrenaline than the renal portion. The ratio was usually between 2-4 : 1, but in one case (a dog) it was even greater, 10 : 1. As these experiments recorded diminution in circumference, they corresponded with an even greater diminution in calibre of the vein *in situ*. The greatest decrease recorded was in the iliac portion of a cat's vein—88 p.c., as compared with 38 p.c. in the renal portion. Such a degree of contraction is rendered even more striking, when one remembers the heavy counterpoise, and it will not do to regard such a vein as a quiescent tube, playing only a passive part in the circulation of the blood. It may be noted, in addition, that on the whole the contraction of the iliac portions lasted longer than that of the renal portions; in no case was the reverse true, and in several the contraction of the renal portion was noticeably brief.

A final test of the comparative contractility of the two portions of the abdominal vena cava was made *in situ* on representatives of all three species, and it fully justified the conclusions already reached as a result of the experiments *in vitro*.

A buck rabbit, weighing 3020 g., was anaesthetized with ether. The superficial leg veins being too small in calibre, the abdomen was opened and a venous cannula inserted into a small tributary at the caudal end of the vena cava. The lower part of the animal was then immersed in a saline bath at body temperature. After it had been a while in this, the board was raised while the external diameter of the vein was measured with a pair of dividers at two fixed points in the iliac and renal portions. The calibres were recorded by pressing the points of the dividers in a sheet of paper. Then the board was again immersed for a while. After this 0.2 c.c. adrenaline 1:1000 diluted with 1.5 c.c. of warm saline was slowly injected through the cannula, and the calibres re-measured twice at intervals after the injection. The measurements so recorded were respectively 4.5 and 11.0 mm., 2.7 and 10.5 mm., and 3.1 and 10.5 mm., so the percentage diminutions in calibre of the two portions of vein were 40 and approximately 5. The animal was then killed with an overdose of ether, and a long upright tube connected with the venous cannula. When this tube was only partly filled with saline at body temperature, the vein had a calibre of 5.5 mm. in the iliac portion, and had not dilated beyond 11 mm. in its renal portion. Nor was there further dilatation on increasing the saline pressure to 160 cm. There was, therefore, a distension limit for the vein, and this was unaffected by removal of the mesentery. Also the calibre of the distended iliac portion was half that of the renal; this sort of ratio was found in the stretched veins of other rabbits. If the diminution in calibre under the action of adrenaline is calculated from the distended calibres, the percentage contractions become approximately 50 and 5.

A female cat, weighing 3600 g., was next anaesthetized with equal parts of ether and chloroform. The superficial leg veins were larger in this animal, and the venous cannula was therefore inserted into the one on the right side. The abdomen was opened, and the calibres of the iliac and renal portions measured before and after an injection of adrenaline diluted with saline, although in this case the injection was washed in with a further 1.5 c.c. of saline in view of the added distance to the vena cava. The figures for this animal were 4.3 and 7.2 mm. before the injection, and 2.5 and 7.0 mm. after it; subsequently the calibre of the iliac portion returned to 4.3 mm. The animal was then killed with an overdose of chloroform, the thoracic inferior vena cava cut open, and the inferior vena cava as a whole washed free from blood. The thoracic end was next clamped off, and the vein subjected to a pressure of warm saline up to 160 cm. After an increase in diameter with the initial rise of pressure to

calibres of 5.6 mm. and 7.6 mm. respectively, the iliac and renal portions alike showed an effective resistance to further distension. The contraction produced by adrenaline in the two portions was therefore approximately 42 and 3 p.c. of their calibre as calculated from the initial figures, or approximately 55 and 3 p.c. calculated from the maximum distension figures.

A dog, weighing 18 kg., and with a snout-buttock length of 99 cm., was anaesthetized with equal parts of ether and chloroform, and, after being used for another purpose, was killed with an overdose of chloroform. The abdominal vena cava was washed clear of blood, and subjected to an increase of internal pressure up to 160 cm. of warm saline, without increase in calibre after a few centimetres pressure. The calibre at two fixed points in the iliac and renal portions was measured at 18 cm. saline pressure before and after an injection of adrenaline, and the percentage diminution of calibre of the iliac portion was just less than twice that of the renal portion.

The fact that the vein soon reached a maximum distension point in all three animals is a justification of the first technique employed for the experiments *in vitro*.

The thoracic inferior vena cava was subjected to *in vitro* tests as a control, although histological examination of this vein from all three species has failed hitherto to reveal any appreciable number of smooth muscle fibres. Sections were fixed in Susa, and thereafter stained with picro-indigo-carmin and orcein. The essential structure of this vein in all three species is collagen and elastic tissue [Franklin, 1931, 1932 *a*], arranged in a special way. Occasionally, with the first technique, a ring from this part of the vein showed a small amount of contraction on immersion in the Ringer's solution at body temperature, but histological examination of a neighbouring ring failed to show more than a very few muscle fibres, quite insufficient in number to account for the contraction. Adrenaline also failed to suggest the presence of smooth muscle. Finally, no effect has ever been shown with the second technique. It may be concluded, from all these observations, that any contraction recorded with this portion of the vein by the use of the first technique was due to warming of the collagen or elastic tissues, or of both.

The relative sizes of circumference in the stretched iliac, renal, and thoracic portions of the inferior vena cava in typical examples of the three species were: rabbit, 10.4, 22.6 and 14.6 mm.; cat, 8.4, 13.4 and 12.0 mm.; dog, 23.2, 34 and 32.6 mm. When these figures are expressed as percentages of the iliac circumference, they become respectively 100,

217, 140; 100, 159, 143; and 100, 147, 141. The relative size of the iliac and thoracic portions seems fairly constant, therefore, in the three species, while the renal portion is comparatively much greater in the rabbit and slightly greater in the cat than it is in the dog. This feature is noticeable on inspection, but only this passing reference will be made to it here, as it demands a more extended investigation. It may also be stated here that the thoracic inferior vena cava of all three species tends to contract, on removal from the body, to a length slightly shorter than its expiratory length with accompanying increase in circumference. The above measurements for the thoracic portions are, therefore, possibly slightly greater than the measurements *in situ* in the expiratory phase of respiration, and markedly greater than those in the extreme inspiratory phase. The experiments on which these conclusions are based will be reported later on completion of the series.

To a considerable degree, then, in the rabbit, to a less but still noticeable degree in the cat, and to only a small degree in the dog, the portion of the inferior vena cava between the renal vein entries and the liver forms a dilatation *en route* to the heart. The reason for this dilatation and for the differences between the three species is at present so far a matter of conjecture that it is not worth while to make any suggestion; the existence of the dilatation and the much greater susceptibility of the more caudal portion of the vein to adrenaline are matters of fact.

Histological examination of the renal and iliac portions of the three species fails to show a sufficiently marked difference in the provision of circular smooth muscle to account for the much greater contractility of the iliac portions, and there are also certain species differences in structure. It may be that in this, as in certain other pharmacological findings, the solution must wait on biochemistry rather than on histology. At all events, it is wiser to defer any histological account until a much larger number of species has been examined. When that has been done, it may be more possible to correlate microscopical structure and physiological action, but in the meantime no useful suggestion can be made from this source.

SUMMARY.

That portion of the inferior vena cava of the dog, cat and rabbit, which lies caudal to the renal vein entries is more reactive to adrenaline than is the portion between these vein entries and the liver. The thoracic inferior vena cava in these species did not give any reaction to adrenaline.

I wish to thank Prof. J. A. Gunn for his advice in connection with this investigation, Dr H. P. Gilding for the supply of some material, and Mr G. K. Elphick for occasional assistance. The expenses of the research have been defrayed by the generosity of the Rockefeller Foundation. The work forms part of a larger study of the venous return in mammals, but is here presented as a separate entity. A similar study of the inferior vena cava of primates is to be made, if possible, under the direction of Prof. J. F. Fulton in the Department of Physiology, Yale University School of Medicine.

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THE MEASUREMENT OF RED CELL VOLUME.

IV. Alterations in cell volume in hypotonic plasma.

BY JOHN MACLEOD AND ERIC PONDER.

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PONDER and SASLOW [1931] showed by means of diffractometric measurements that the volumes assumed by red cells in hypotonic solutions of various salts are much less than those which would be expected on the supposition that the cells behave as "perfect osmometers," and accounted for the results by assuming that osmotically active substances are lost into the suspension media (hypotonic NaCl, KCl, and glucose). They attempted, also, to establish the same result for red cells suspended in hypotonic plasma, but not with conspicuous success, for the only method at their disposal for the measurement of the volumes in hypotonic plasma was the hæmatocrit method, which is not reliable [Ponder and Saslow, 1931]. The volume changes which the cells undergo in hypotonic plasma, however, are particularly important, for at least two reasons.

(1) The volumes attained in solutions of NaCl, KCl, and glucose, of various tonicities, were found from the diffractometric measurement of the radius of spherical forms. There is no doubt that the diffractometric measurements themselves are reliable [see Ponder, 1932], but the objection can always be raised that the same kind of forces which render the cells spherical when between a slide and cover glass alter their semi-permeability and render them liable to lose osmotically active substances.

(2) It can also be objected that changes of pH may occur when red cells are suspended in NaCl, and particularly in glucose. More specifically, this type of objection is related to the recent conclusions of Jacobs and Parpart [1932], who account for many of the phenomena of osmotic hæmolysis in terms of changes in the base-binding power of hæmoglobin. Jacobs and Parpart give their conclusion in the form of an equation,

$$\frac{W_1}{W_2} = \frac{2R+1-F_1}{2R+1-F_2}, \quad \dots\dots(1)$$

in which R represents the ratio of base in the cells to the cell hæmoglobin, F_1 and F_2 the amounts of base bound by unit quantity of hæmoglobin under two conditions corresponding to the presence of quantities of water W_1 and W_2 in the cell (*e.g.* two different pH 's), and where the concentration of salt C in the suspension medium is constant: if F is constant, however, and C is varied,

$$\frac{W_1}{W_2} = \frac{C_2}{C_1}, \quad \dots\dots(2)$$

i.e. the cell behaves as a "perfect osmometer." They find that expression (1) satisfactorily describes the results of those experiments in which F_1 and F_2 are varied by changing the pH , the temperature, or the degree of oxygenation of the hæmolytic system, and conclude that, although the existence of factors "other than purely osmotic ones" is by no means excluded, such factors are probably of secondary importance and better disregarded until further calculations based on better data have been made.

It is therefore important to examine the behaviour of erythrocytes in hypotonic plasma rather than in hypotonic saline, for in the latter medium the abnormally small volumes observed might conceivably be due to the operation of such factors as Jacobs and Parpart discuss (particularly pH changes), while in the former medium such factors are constant and expression (2), which treats the cells as perfect osmometers, ought to apply. A difficulty arises, however, when we come to look for a method of measuring the volumes directly. The colorimetric method [Ponder and Saslow, 1930] cannot be used for very hypotonic solutions without becoming exceedingly complicated; the hæmatocrit method is not reliable, and the diffractometric method is not applicable to cells in plasma, which are discoidal. We have accordingly used three methods of quite a different character, all based on very simple principles.

I. METHODS.

(a) *Red cell density.*

The principle of this method is that the volume of water taken in by the red cells, and therefore their increase in volume in a hypotonic solution, can be found from the values for their density before and after such swelling. The percentage increase in volume being known, the amount of "free water" (erroneously regarded as such) contained in the cells can be calculated on the assumption that water exchange is the only factor concerned in the swelling and that the cells behave as perfect

osmometers. The extent to which this figure for the "free water" departs from the true value (about 95 p.c. of the total water) enables us to judge the extent to which the cells depart from being "perfect osmometers" by losing osmotically active substances into the surrounding medium [see Ponder and Saslow, 1931].

Rabbit blood is obtained from the carotids, potassium oxalate being used as an anti-coagulant. The percentage volume of the cells present is found by the hæmatocrit, the tubes being spun at 4000 r.p.m. for 15 min. The density of the cells, suspended in their own plasma, is found by the method described elsewhere by one of us [MacLeod, 1932]. This determination alone requires about 15 c.c. of blood.

About 25 c.c. of the whole blood is set aside, and plasma is obtained from the remainder. The tonicity of this is taken as unity, thereby avoiding difficulties attendant on the conversion of the tonicity into an equivalent solution of NaCl. The plasma is then diluted with distilled water to give a hypotonic plasma, *e.g.* if a final tonicity of about 0.7 is required, 70 c.c. of plasma is diluted with 30 c.c. of water. It is convenient to make the total volume of the diluted plasma 100 c.c. This volume is then added to 20 c.c. of the whole blood and the mixture allowed to stand for a few minutes: all operations should be carried out at constant temperature, which, in the case of these experiments, was 27°. The 120 c.c. of mixed blood and plasma is now transferred to centrifuge tubes, the cells packed, and their new density found as before. This new density is always less than the initial value, for the cells have taken in water.

The tonicity of the 100 c.c. of diluted plasma is, of course, increased by the addition of the plasma contained in the 20 c.c. of added blood, but the new tonicity of the medium can easily be calculated. The assumption is now made that the cells, originally in equilibrium with plasma at a tonicity of unity, take in water so as to come into equilibrium with the diluted plasma surrounding them; the quantity of water which 100 c.c. of cells must gain in order to do so is then given by

$$x = \frac{100 (d_1 - d_2)}{d_2 - 1}, \quad \dots\dots(3)$$

where d_1 is the initial density and d_2 the density after the swelling has occurred. The volume thus increases from 100 to $100 + x$, and so the percentage increase in volume can be found.

A very small correction is now applied to the figure for the tonicity of the diluted plasma, for a quantity of water has been lost from it and has passed into the cells. Call the corrected tonicity T and the quantity of

water transferred to the volume of cells present in the particular experiment x' . Then the apparent quantity of water W_1 in the cells is

$$W_1 = Tx'/(1 - T). \quad \dots\dots(4)$$

Finally, the actual quantity of water present in the cells, W_2 , is found by weighing a quantity of packed cells before and after drying at 60° [see MacLeod, 1932]. The fraction of the total water which is "free" is $R = W_1/W_2$.

The only significant sources of error in this method are those associated with the density determinations. Even if we allow an error of -0.001 in d_1 and an error of $+0.001$ in d_2 , the error introduced into the value of x only amounts to about 3 c.c. in 20 c.c., *i.e.* a volume increase of 20 p.c. becomes one of 17 p.c. This leads to an under-estimation of R , in a typical experiment, of about 0.10, but, since such errors in the density determinations are unlikely to occur, and since the difference between the behaviour of the cells and that of perfect osmometers is far greater than could be accounted for by such an error in the evaluation of R , the method is adequate for our purpose.

(b) *Hæmoglobin determinations.*

The principle of this method is that the hæmoglobin content of unit volume of packed cells is reduced if the cells take in water, and that the amount of water so taken in can be calculated from the extent of such dilution.

Blood is obtained as in the first method and the percentage volume of the cells determined. The cells of about 5 c.c. of blood are packed in a rapid centrifuge, the supernatant fluid carefully removed, and the percentage volume of the packed cells found by hæmatocrit. Usually the percentage volume is more than 95 p.c. With the greatest care, 1 c.c. of the packed cells is removed and added to 1000 c.c. of 1 p.c. HCl. This constitutes the "standard."

About 25 c.c. of plasma is obtained, and its tonicity denoted by 1.0, as before. This is suitably diluted with water so as to give 25 c.c. of hypotonic plasma, which is then added to 5 c.c. of whole blood. After a few minutes the mixture is centrifuged and the supernatant plasma removed. The percentage volume of the packed cells is again determined: 1 c.c. of the packed cells is then added to 1000 c.c. of 1 p.c. HCl. This constitutes the "unknown."

The standard and the unknown are matched against each other in a colorimeter with a Newcomer eyepiece, and the quantity of added water

calculated from the degree of hæmoglobin dilution in the unknown. Corrections of an obvious nature are made for the increase in the tonicity of the hypotonic plasma by the addition of the plasma contained in the added blood, and for differences between the percentage volume of the packed cells obtained from normal plasma and hypotonic plasma respectively. The remainder of the calculations are the same as in the first method.

This method has about the same degree of accuracy as the density method, the principal errors being those associated with the estimation of the percentage volume of the packed cells and with the transference of the 1 c.c. of packed cells from normal and hypotonic plasma respectively. With care the latter can be made quite small, although it is always difficult to transfer such viscous material with great accuracy.

(c) *Total water.*

Just as the total quantity of water contained per unit volume can be determined for cells taken from normal plasma, so can it be determined for cells which have been immersed in hypotonic plasma. The methods used for finding the total water present are those described by MacLeod [1932], the water being driven off at 60° from cells from normal and hypotonic plasma respectively, until constant weight is reached in each case. The cells from hypotonic plasma, of course, always contain a greater percentage of water. The calculations are made along obvious lines, similar to those employed in the two foregoing methods.

II. RESULTS.

(a) *Cell density.*

The following results are typical of those obtained.

T	d_1	d_2	W_1	W_2	R
0.646	1.0979	1.0820	1.78	3.21	0.55
0.645	1.0993	1.0822	2.88	4.63	0.62
0.734	1.0969	1.0856	2.76	4.25	0.65
0.846	1.0994	1.0918	3.00	4.16	0.72

The values of R lie between 0.55 and 0.72, as in experiments in hypotonic saline and hypotonic plasma (diffractometric and hæmatocrit determinations, Ponder and Saslow [1931], and as in Warburg and Winge's hæmatocrit determinations in hypotonic plasma). It is clear that in this latter medium, despite the constancy of pH , etc., the cells do not behave as "perfect osmometers," but lose osmotically active substances into the surrounding fluid, as has, indeed, been shown by the direct analyses of Kerr [1929].

(b) *Hæmoglobin determinations.*

This method gives substantially the same results as the foregoing. For example,

T	x	x'	W_1	W_2	R
0.646	23.0	0.437	0.08	1.24	0.64
0.732	16.1	0.322	0.88	1.26	0.69
0.829	11.1	0.200	0.97	1.70	0.57
0.863	7.8	0.164	1.03	1.95	0.53

Again R varies between 0.53 and 0.69, and we are led to the same conclusion as before.

(c) *Total water.*

The results of the total water determinations agree remarkably closely with those found by the density method. This can be seen from the following table, which gives the percentage increase in volume in media of various tonicities, as obtained by the two methods.

T	x	
	method (a)	method (c)
0.646	19.3	19.1
0.645	21.0	22.5
0.734	15.5	15.2
0.846	8.5	8.6

These results are of particular significance because the method used is so simple and direct. Consider, for example, the case where $T = 0.646$, and where the cells in isotonic plasma contain 66 p.c. of water. If these were brought into osmotic equilibrium with hypotonic plasma at $T = 0.646$, their volume would increase from 100 to 136 p.c.; if an exchange of water alone were responsible for the volume increase, we would expect the value of x to be 36 p.c. The fact that it is always about half this value constitutes the strongest argument against the behaviour of the cell as a "perfect osmometer."

III. DISCUSSION.

Taking these results together with those published in previous papers, we can draw the following conclusions.

1. Every method by which volume measurements have been made, whether directly or indirectly, leads to the same general conclusion, viz. that the rabbit red cell swells less in hypotonic media than it would if water exchange alone were involved. The evidence on this point is very strong (colorimetric determinations, Ponder and Saslow [1930]; diffractometric determinations, Ponder and Saslow [1931]; hæmatocrit

determinations, Ponder and Saslow [1931], Warburg and Winge [1928]; and the determinations by the three methods described in this paper). Taken together with the results of Kerr [1929], these results force one to the conclusion that the cells lose osmotically active substances into the surrounding hypotonic fluid.

2. There is no essential difference between the results obtained in hypotonic NaCl and KCl and those obtained in hypotonic plasma. This disposes of the possible objection that the abnormally small amount of swelling is primarily due to *pH* changes occurring in the former media.

3. It follows from the preceding that expression (2), as given by Jacobs and Parpart, is incorrect, for it states that the swelling in hypotonic solutions can be accounted for by water exchange alone if *pH*, temperature, degree of oxygenation, and other factors affecting the base-binding power of hæmoglobin are kept constant. But direct measurements of cell volume show that this is not the case, even in hypotonic plasma in which *pH*, temperature, and the other factors are kept constant as required. Further, if expression (2) is incorrect, expression (1) must be incorrect also, for it is based on the same assumptions. There is no doubt, of course, that changes in the base-binding power of hæmoglobin affect the osmotic pressure of the cell interior and therefore the figure for the tonicity which produces hæmolysis, and that they operate in the direction which Jacobs and Parpart indicate; it is impossible, however, to express their effects in quantitative form until we are able to express, with quantitative exactness, the effect of the loss of osmotically active substances from the cell¹.

4. In spite of its relatively indirect nature, the diffractometric method of measuring red cell volume seems to yield essentially the same results as do other and more direct methods. This conclusion is of considerable importance from a purely technical point of view.

¹ Jacobs and Parpart claim that expression (1) is quite satisfactory for describing results which they have obtained in experiments in which *pH* and other factors were varied. But the experimental verification of their equation rests, not on direct volume measurements, but on an assumption which may well turn out to be incorrect, viz. that the cells hæmolyse at exactly the same critical volume, irrespective of the *pH*, etc., of the hypotonic medium in which they are placed. The agreement between their experimental and calculated results, moreover, is only to "the same order of magnitude," which is scarcely sufficient.

SUMMARY.

The quantity of water taken up by red cells when immersed in plasma of varying degrees of hypotonicity is found by three methods: (a) by determining the decrease in density of the cells consequent on this intake of water, (b) by measuring the diminution in hæmoglobin content of the swollen cells, and (c) by determining the quantity of water taken in by drying the cells and weighing. It is shown that the amount of water taken up by the cells as they swell is much less than that which would be expected if the cells were "perfect osmometers," *i.e.* if they took in water without losing osmotically active substances into the surrounding hypotonic medium. This is the same kind of result as has already been obtained for cells immersed in hypotonic NaCl, KCl, and glucose.

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THE INVESTIGATION OF THE TOXIC EFFECTS OF LARGE AMOUNTS OF SUGAR IN THE BLOOD.

By COURTENAY EVANS.

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Medical School.)*

MANY experiments have been recorded in the past in which toxic effects are ascribed to the introduction of large amounts of sugar in the blood. On the other hand, observations have also been repeatedly made in which no such symptoms could be detected as a result of this procedure.

Among the former may be mentioned Harley [1893] who describes in dogs tremors, acetonuria, exaggerated respiratory movements, convulsions and coma; and Kossa [1899, 1911] who noted in fowls muscular weakness and inco-ordination. Allen [1913] who reviews the whole subject adds deaths from lowered resistance in guinea-pigs and rabbits, and anorexia and ataxia in dogs and cats; while Williams and Swett [1922] and Stoddart [1924] describe rigor and fever when unbuffered solutions are used.

None of these or any other toxic symptoms were observed by Kleiner and Metzger [1913, 1914], Albritton [1924], Boyd, Hines and Leese [1925], La Barre and de Cespédès [1931], in dogs, nor by Lamy and Mayer [1904] in dogs and rabbits, nor by Jüttemann [1930] in guinea-pigs, nor by Rigler and Uhrich [1923], Thalhimer, Raine, Perry and Buttlers [1926], Lennox and Bellinger [1927] in man.

In few of these experiments were high concentrations of blood sugar recorded, and Harley [1893] with a reading of 676 mg. per 100 c.c. and Kleiner and Metzger [1913, 1914] with readings of 720 mg. in normal and 1100 mg. per 100 c.c. in depancreatized dogs are the observers recording the greatest sugar concentration. In order to re-investigate this matter, large quantities of glucose were injected intravenously in normal rabbits with the object of observing any acute, toxic or nervous phenomena as the result of high concentration of sugar in the blood.

DESCRIPTION OF EXPERIMENTS.

Varying amounts of glucose solution 10 and 15 p.c. in sterile distilled water were injected intravenously into the veins of one ear of a rabbit. Samples of blood were taken from the veins of the other ear. Normal rabbits with large ear veins were used, no anæsthetic was administered, and the time of injection was on an average 20 minutes. The ears were shaved and a fine "Record" needle attached to a syringe (10 or 20 c.c.) inserted in a vein, and held in position by an assistant. The animals were placed in a box which fitted them comfortably, for convenience of handling during the experiment.

The amounts of sugar in the blood were estimated by Hagedorn and Jensen's method. Certain modifications had to be carried out, as 2 c.c. of potassium ferricyanide are completely reduced when the percentage of sugar is above 385 mg./100 c.c. In the first six experiments, 0.1 c.c. of blood was taken and further amounts of 2 c.c. of potassium ferricyanide were added as the solution became colourless on boiling; and several blank determinations were made according to whether 2, 4, 6 or 8 c.c. were used. In the last three experiments, in order to lessen the volume of fluid at the final titration, smaller amounts of blood were taken when it was suspected that the sugar content was high. In the seventh experiment duplicate estimations were carried out, using 0.025 and 0.05 c.c. of blood respectively, and the results agreed fairly accurately, 1044 and 1060 mg./100 c.c.

RESULTS OF EXPERIMENTS.

(1) *Blood sugar curves.*

The first readings prior to injections showed a normal value for blood sugar. No readings were taken during injection. Following the injection high blood-sugar values were found. In the earlier experiments less amounts of glucose were injected and the readings were 600 to 900 mg./100 c.c., in the later experiments when more glucose was injected values of 1300 to 1900 mg. were obtained. The blood sugar fell rapidly, so that in most cases the level was normal again in 3 hours. In the last experiment when the blood-sugar level of 1900 mg./100 c.c. was reached, the level had only fallen to 236 mg. in 4 hours. In one experiment the sugar in the blood from the animal's heart was found to be 1380 mg./100 c.c., a reading similar to that which would have been expected from blood in the ear.

TABLE I.

Exp.	Animals, weight, date	Glucose injected			Duration of injection min.	Time of samples after injection min.	Blood sugar values in mg./100 c.c.	Comments
		Strength of solu- tions p.c.	In c.c. of solu- tion	In g.				
1	Female 2 kg. 12. viii. 31*	10	80	8	30-45	Before	129	Urine sugar + + present
						3	627	
						5	480	
						20	466	
2	Male 2 kg. 13. viii. 31	10	120	12	—	4	953	Control blood sugar 122. Urine sugar + +
						5	876	
						10	762	
						40	561	
						85	242	
3	Female 13. viii. 31	10	45	4.5	—	140	171	
						Before	125	
						5	595	
						8	563	
						10	530	
						40	421	
						90	134	
						165	130	
4	7. i. 32	10	110	11	20	Before	64	
						1	887	
						10	684	
5	2 kg. 11. i. 32	15	90	13.5	10	Before	140	
						1	1051	
						2	892	
						12	660	
6	19. i. 32	15	150	22.5	40	35	638	
						Before	131	
						2	1335	
						20	911	
						55	728	
7	2. ii. 32	15	90	13.5	40	125	565	
						Before	89	
						1	1052	
						170	300	
8	9. ii. 32	15	180	27	20	Before	91	
						1	1616	
						25	988	
9	16. ii. 32	15	200	30	20	180	132	
						Before	107	
						1	1902	
						25	> 1100*	
						235	236	

* Tube broke during experiment.

(2) Effect on rabbit.

Large quantities of urine were passed which reduced Benedict's solution; the rabbits ran about the laboratory immediately after the injection, and there was no evidence of inco-ordination of movement, weakness of muscles, or coma. The hair of the rabbits remained good, and no trophic lesions were noted, except occasionally when a hæmatoma had occurred at the seat of injection, or sugar solution had passed into the subcutaneous tissue. No chills or rigors were noted; the rabbits did not always feed well for a few days following the injection. One animal became pregnant during the course of the experiments and later gave birth to a litter of seven, four of which survived and are now healthy rabbits. This was an unintentional result, since it was not known that the rabbit was pregnant. The two rabbits on which the last six experiments were carried out were alive and well 6 months after the last injection.

One of the rabbits was killed 7 months after the last injection and a post-mortem examination made. No abnormality was seen on macroscopic examination of heart, brain, pancreas, kidneys, suprarenals, liver, spleen, testes and blood vessels. The lens of the eye was crystal clear. Microscopic examination of the pancreas, spleen, aorta, suprarenals, kidneys, heart muscle and testes showed no abnormality. The liver also appeared normal, but only very small amounts of glycogen were demonstrated histologically.

DISCUSSIONS.

In these experiments it was intended to observe any acute toxic effects of high concentrations of sugar in the blood. With the exception of one animal that died (and which was found to have a dilated heart) none were seen. No toxic after-effects became apparent. Higher blood-sugar concentrations were achieved than have been previously described in the literature. The blood-sugar value rapidly fell and the longest period of hyperglycæmia (1900 falling to 230 mg./100 c.c.) was 4 hours.

SUMMARY.

1. Experiments are described in which large quantities of glucose were injected into the blood stream and very high readings of sugar concentration are recorded.

2. No pathological effects could be attributed to the raised blood sugar.

The experiments were carried out at the Physiological Department, St Bartholomew's Hospital Medical College. I wish to thank Prof. Hartridge and Dr Gordon Reeves for their assistance in preparation of this paper, and I am indebted to Dr George Graham and Dr Harrison for helpful suggestions concerning the experiments.

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THE RATE OF BLOOD FLOW AND GASEOUS METABOLISM OF THE UTERUS DURING PREGNANCY.

By J. BARCROFT, W. HERKEL AND S. HILL.

(From the Physiological Laboratory, Cambridge.)

BARCROFT and ROTHSCILD [1932] found that the uterine vessels of the rabbit contained, towards the end of pregnancy, large amounts of blood, sometimes as much as 30 c.c. An answer to the question whether this blood is being stored or is in active circulation demands measurements of the quantity of blood passing through the uterus per minute, and any further discussion requires some knowledge of the extent to which that blood is utilized by the uterus. The present paper describes an enquiry into these problems, but deals only with the period between the fourteenth and twenty-eighth days of intra-uterine life—that is through the period of growth of the foetuses from the time when they begin to be weighable. The last two days of pregnancy present some rather baffling problems, which must be the subject of further enquiry.

THE VOLUME OF BLOOD WHICH TRAVERSES THE UTERUS.

The literature appears to contain no data with regard to the quantity of blood which flows through the uterus per minute.

DISSECTIONS.

The blood vessels in the rabbit, which lead from the generative tract, have been described in a former paper [Barcroft and Rothschild, 1932]. After testing several procedures we settled down to the following as being one which enabled us to collect all the blood from the uterus (though not from the ovaries, Fallopian tubes or vagina) without serious prejudice to the blood-pressure. The rabbit was given dial (about 0.6 c.c. per kg.). This hypnotic though in many ways satisfactory is uncertain as regards dosage; if the dose indicated proved insufficient, some ether was given as well. (Another rabbit to be used as a donor was similarly anaesthetized and a cannula inserted into its carotid artery.)

The dissections were carried out in the following order:

(1) A cannula was tied in the right external jugular vein for the reception of blood from the donor.

(2) A cannula was tied in the left carotid for the blood-pressure tracing.

(3) A small incision was made in the right side through which the right ovary was withdrawn and tied off together with the tissue in which it was embedded. The ligature included not only the ovary but also the Fallopian tube and incidentally the vascular connections of the genital tract *via* the right ovarian vessels. The incision was then sewn up.

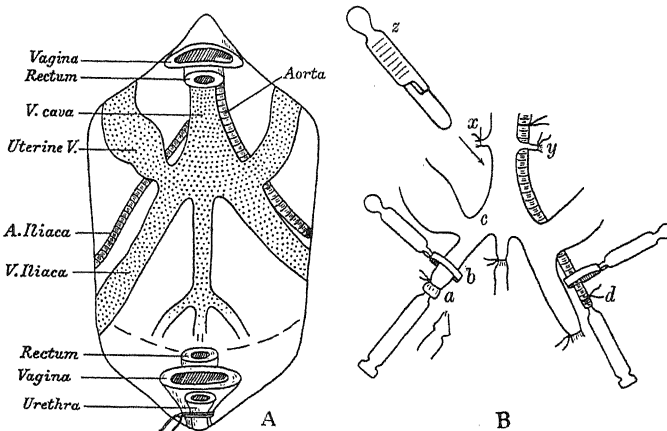


Fig. 1. A, General disposition of vessels as seen after removal of bladder, rectum and vagina. B, dissection showing the vessels tied and clipped, and the cannulae inserted.

(4) The left ovary, Fallopian tube and vessels were treated similarly.

(5) A median incision was made over the bladder. After tying the ureters and the necessary vessels on the surfaces of the bladder and vagina a ligature was passed round the urethra, the rectum and the lower part of the vagina; this was firmly tied. Another ligature was passed and tied round the rectum and the upper portion of the vagina just below its junction with the uterus. The bladder, vagina and rectum were then cut out.

Through the window so made the veins appear (after clearing up the overlying tissue) as in Fig. 1 A. Our first efforts were in the direction of using one side of the uterus only, by inserting a cannula for the collection of the blood at *a*, Fig. 1 B, and, when we wished to collect the uterine blood, removing the clip from *b* to *c*. This procedure did not

succeed because the artery and the vein at *c* are often so nearly inseparable that the artery was interfered with by the clip, if not actually included in it. We therefore settled down to the following dissection which was carried out in the order here given. The coccygeal artery and vein were tied with a single ligature. The small veins *x* and *y* were tied and cut; according to the exigencies of the case they were tied either with one ligature or with two. The arteries corresponding were usually, but not always, treated in the same way. The vena cava was separated from the aorta and a loop of thread passed loosely round the latter. The aorta could then be drawn a little to one side without stopping the blood flow. Thus it was possible completely to explore the vena cava and dissect all tissue away from it, so that when the time came for the collection of a sample, a bull-dog forceps *z* could be placed on the vena cava at the point indicated by the arrow. This bull-dog completely occluded the vena cava without interfering with the aorta.

(6) The iliac vessels were dissected out on the left side, from below Poupart's ligament upwards, the iliac vein was tied and a cannula was tied into the artery, *d*.

(7) Similar dissections were made on the right side, but here the artery and its tributaries were tied and the cannula was put into the vein at *a*. It is necessary that this cannula should offer as little resistance as possible. The smallest one used in our experiments, together with the attached tubing, allowed 30 c.c. of water to pass per minute with a pressure head of 6 cm. of water. Measurements showed that in the later stages of pregnancy the blood-pressure at *c* was about 6 cm. of blood, *i.e.* if an open tube were attached to the cannula the open end had to be raised 6 cm. above the point *a* in order to prevent the blood from flowing out. It appeared therefore that if the blood flow was 30 c.c. a minute the cannula would introduce approximately the correct resistance. If the blood flow were less than this the veins might be expected to empty themselves a little before a steady flow through the cannula took place—for this reason in collecting a sample a little blood was usually allowed to go to waste between the moment of putting the bull-dog on the vena cava and collecting the blood for analysis. When the dissections were complete heparine was injected—75 mg. of the powder supplied by Messrs Hynson, Westcott and Dunning of Baltimore in 10 c.c. of Ringer was the usual amount. In some of our latter experiments we used the fluid preparation of Jacobi which we found to be equally satisfactory. The registration of arterial pressure was then begun and blood passed into the jugular vein from the carotid of the donor.

The collection of blood samples.

Everything was now ready for the collection of blood samples, and this was done as rapidly as possible; the times at which the samples were collected were registered approximately on the blood-pressure tracing, and the actual intervals during which the venous samples were collected were measured with a stop-watch.

The usual routine was to withdraw an arterial sample of about 3 c.c., put it under paraffin and then collect three venous samples in rapid succession.

The venous samples were obtained as follows:

(1) The clip at *b* was removed and the rubber tubing attached to the cannula was held with its open end at a level sufficiently high to prevent the blood from coming out.

(2) The clip *z* was put on the vena cava and the end of the rubber tubing lowered to a level just below that of the vein. Now all the blood from the uterus and none, or a negligible quantity, from elsewhere was flowing down the tubing. After a little blood had escaped, the open end of the rubber tubing was placed under the surface of some liquid paraffin in a separating funnel and blood was collected for, say, half a minute; at once a second similar sample was collected and then a third. The times were not half a minute in all cases; in the rabbits at early stages of pregnancy, and in which the blood flow was slow, "minute samples" were taken and in some experiments the time interval was $\frac{2}{3}$ of a minute. Our aim was to obtain some sort of equality in the quantities of blood withdrawn. It is questionable whether our policy was the best. It would have been simpler and possibly no worse to have adhered to half-minute samples throughout.

It may be desirable here to touch upon our greatest source of solicitude, that of being justified in the belief that the rates of flow which we measured really represented those in the animal. The points for consideration were:

(1) That between the actual capillaries of the uterus and the cannula there is a large venous bed in which a considerable quantity of blood could accumulate or from which it could drain. Behind our measurement lies the assumption that the quantity of blood in the venous bed does not alter to any significant extent whilst we are collecting our samples. The assumption so made is probably correct when the quantity of blood collected in the first half-minute is the same as that collected in the second, for it is unlikely that the venous reservoir would fill or drain at

a constant rate over so long a time. In the main, we had been successful in securing a pretty constant rate of flow over the first two periods of collection.

(2) The withdrawal of blood naturally tends to lower the general arterial pressure and slow the blood flow through the uterus. In our earliest experiments we endeavoured to replace the blood withdrawn by defibrinated blood injected into the jugular vein. This technique was disappointing. Possibly we should have been more successful had we tried the injection of heparine blood.

The method which we adopted was as follows. At the end of our dissection the blood-pressure was usually about 90 mm. The rabbit was then supplied with blood from the donor which raised the pressure somewhat at first. After the pressure reached 110–120 mm. there was little or no further rise even though more blood was run in; it seemed probable therefore that this extra quantity of blood could be withdrawn without any considerable fall of blood-pressure.

In practice we found that when we were taking our venous samples, the first two samples caused very little alteration in general arterial pressure but that the drop became more marked during the withdrawal of the third sample, and the blood flow correspondingly slower.

In the following discussion we adopted the plan of accepting the figures for the first minute of collection. This decision was of course arbitrary. Frankly it must be admitted that such a course does not exclude considerable errors. Our principal justification is that we tried several methods for the calculation of our results and they all gave the same general picture. It is only this general picture which we would stress.

A rather attractive method was that of accepting the rates of blood flow for the second half-minute only, on the ground that those for the first half-minute were more likely to be vitiated by alterations in the venous reservoir, whilst those in the third half-minute were more likely to be vitiated by alterations in the general arterial pressure. Having accepted the rates of blood flow for the second half-minute, the gas analyses for the third half-minute could be combined with them on the ground that the blood collected in the third half-minute was that which actually traversed the capillaries during the second. This procedure seemed complicated and suggestive of a claim to accuracy which we do not wish to make.

RESULTS.

Fig. 2 shows the rate of blood flow from the vein plotted against the period of embryonic life which has been reached. The maximum rate of flow is about 30 c.c. per minute.

The comparison of the results shown in Fig. 2 with those of Fig. 4 in a recent paper by Barcroft and Rothschild [1932] yields some points of interest.

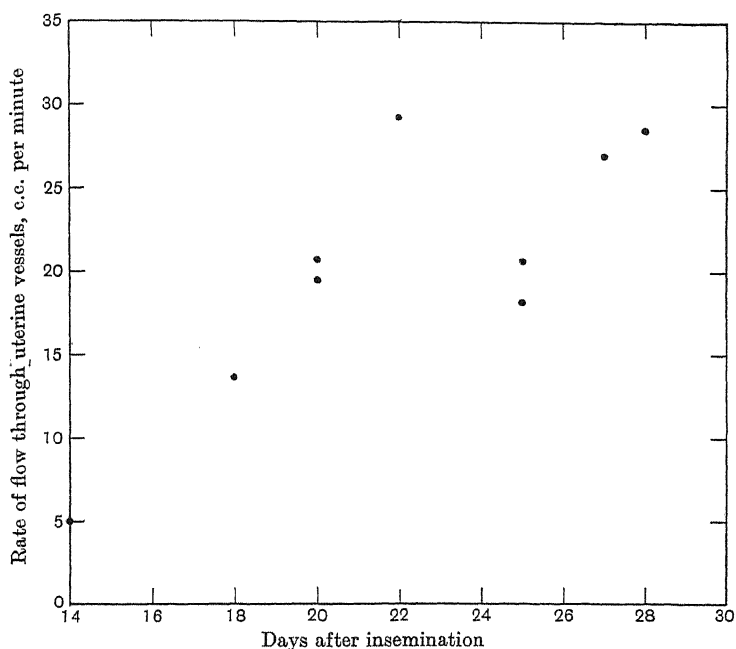


Fig. 2. Rate of flow of blood through the uterus from the fourteenth to the twenty-eighth day of pregnancy.

(1) The amount of blood which traverses the uterus per minute is of the same order as the volume of blood in the organ at any one time. In this the uterus appears to occupy a position intermediate between say the kidney in which the amount of blood in the organ at a given time is small relative to the amount which traverses it per minute, and the spleen where, at times, there is a large volume of blood that is normally almost stagnant. This relatively slow flow is doubtless due to the great development of veins in the broad ligament.

(2) The values obtained for this blood flow confirm those obtained for the blood content in showing that the quantitative vascular changes take place in advance of the embryonic growth. Thus as early as the fourteenth day the blood flow is quite rapid, 5 c.c. per minute, whilst by the eighteenth or nineteenth day it has reached 15 c.c. per minute, about half the maximum; yet on the eighteenth day the embryos are perhaps only a twentieth of their ultimate weight.

(3) We may emphasize the fact that the quantity of blood which emerges from the uterus appears to be less than that which is supplied to it. This fact was revealed by measurements of the oxygen (or CO) capacity of the venous blood and of the arterial blood, respectively.

The following data were obtained.

Rabbit	Day after insemination	CO-capacity (vol. p.c.)	
		Venous	Arterial
64	22	15.0	13.4
70	28	16.7	14.0
74	29	14.7	13.3
76	29	17.5	15.3

The above data represent the facts; their explanation is obscure and must await further investigation. The higher concentration of the uterine venous blood may be due in part to considerable lymph formation.

During the twenty-ninth and thirtieth days of pregnancy we obtained very variable results. In some experiments we obtained markedly low results, in others, results comparable with those obtained on the twenty-eighth day. The reasons for the disparity are not at present clear. Two considerations must be borne in mind.

(1) In counting the days the essential factor, at this very late stage, is not the number of days after insemination, but the number of days before birth. If there is really a fall in the blood flow before birth, this for example might appear on the twenty-ninth day if the young were to be born on the thirtieth and not till the thirtieth day if birth was to take place on the thirty-first. Hence variable results for the twenty-ninth day might be obtained.

(2) It is possible that shortly before birth the blood flow waxes and wanes. We made some observations of the pressure at the junction of the iliac and uterine veins during birth itself. The pressure rose with each peristalsis, fluctuating between 3 and 13 cm. of blood. At what stage this fluctuation first appears we do not know.

The oxygen metabolism of the pregnant uterus.

Fig. 3 shows the quantity of oxygen used by the uterus according to our very crude calculations. The figures are those for the first minute of our observations. They are obtained by subtracting the oxygen content of 1 c.c. of venous blood drawn in the first minute from that of 1 c.c. of arterial blood and multiplying the result by the average rate of flow during that period.

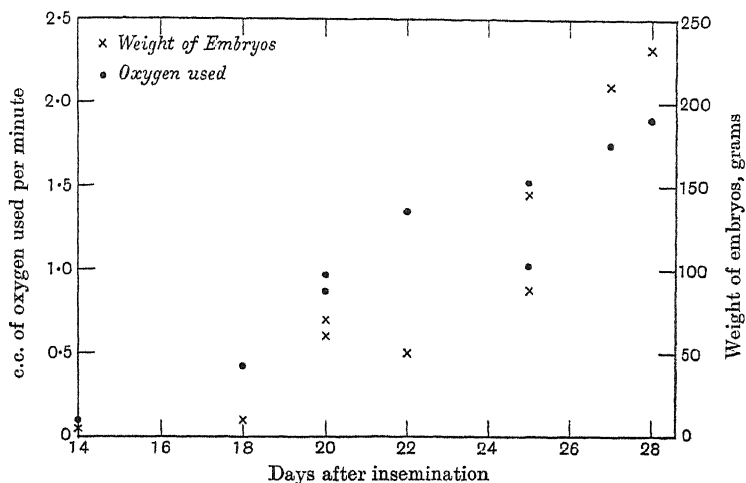


Fig. 3. Oxygen used by pregnant uterus and contents compared with aggregate weight of foetuses.

The principal sources of error, and they are considerable, are :

- (1) The blood collected during the first minute was not necessarily that which traversed the capillaries during that time interval.
- (2) The uncertainty of the blood flow during the first half-minute owing to the large venous reservoir.
- (3) The fact that no allowance has been made for the concentration of the blood.

Yet great as are these sources of error any variation which they cause would not alter the large features of the picture:

- (1) That the oxygen usage up to about the eighteenth day is trivial, being under 0.1 c.c. per minute.
- (2) That between the eighteenth and twenty-second days it increases rapidly, up to about 1.35 c.c. per minute.
- (3) That between the eighteenth and twentieth days it undergoes sudden increase, attaining values of about 1 c.c. per minute.

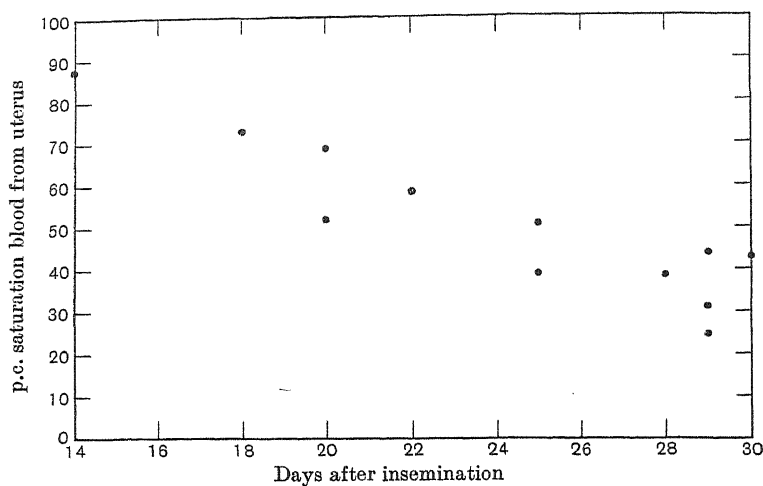


Fig. 4. Percentage saturation of blood in uterine veins from the fourteenth to the twenty-eighth day of pregnancy.

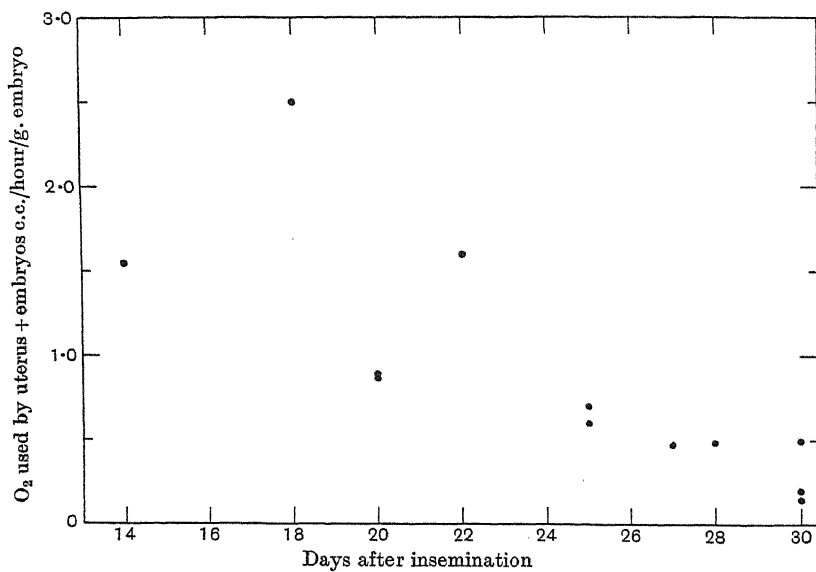


Fig. 5. Ordinate: oxygen consumption of uterine contents divided by weight of embryos. Abscissa: days after insemination.

(4) That after the twenty-second day there is a gradual increase, the ultimate figure being about 2 c.c. per minute.

As the increase in the size of the uterus between the eighteenth and twentieth days is not very remarkable, we may conclude that after the eighteenth day the metabolism of the uterus itself forms a negligible factor in the total which is almost entirely attributable to the embryos¹. The significance then of the great rise in oxygen metabolism between the eighteenth and twentieth days is due to the sudden growth of embryonic tissue. Before this date it is true that the metabolism of the embryo per gram of material may have been as great or greater, but the embryos are so minute that the absolute quantity of oxygen which they use is insignificant.

From the twentieth day onwards to the twenty-eighth the oxygen used does not increase in the same ratio as the weight of the foetuses. In spite of the error introduced by neglecting the oxygen uptake by uterus and placenta, which of course accounts for a much larger proportion of the oxygen used in the earlier stages, Fig. 5 shows clearly that there is a decrease in metabolic rate with age. Thus the rate of embryonic metabolism in the rabbit varies in the same way as that of the developing guinea-pig [Bohr, 1900] and chick [Bohr and Hasselbalch, 1900; Murray, 1926; Hanan, 1928].

The saturation of the venous blood.

The facts: (1) that the vascular conditions are established largely before the embryos are of appreciable size, and (2) that the embryos in the last fortnight grow out of all proportion to the vascular bed, explain the fact that starting from over 90 p.c. saturation the oxygen in the venous blood steadily falls till at the end of pregnancy saturation drops to between 45 and 25 p.c. (Fig. 4).

If as a first approximation one views the circulation in the uterus as consisting of bloods of very different oxygen tensions reaching the organ (*i.e.* the bloods in the uterine and umbilical arteries) and bloods approximately in equilibrium leaving it (*i.e.* the bloods in the uterine and umbilical veins), we arrive at a conclusion which is in line with those of previous authors who have made direct analyses of the blood in the umbilical vessels. Cohnstein and Zuntz [1884], Huggett [1927] and Haselhorst and Stromberger [1930] showed that the tension of oxygen in the blood which supplies the foetuses is very low, 20–30 mm. Hg.

¹ More recent observations by one of us (Herkel) suggest that the metabolism of the uterus itself during pregnancy is greater than we had supposed.

When we get as low as 20 mm. we are getting to a critical point on the dissociation curve. Between 60 and 20 mm. pressure large changes in saturation take place with small changes in pressure, but any reduction of the blood below the point at which we have arrived means a drop in pressure at an ever-increasing rate. If the two blood streams which leave the uterus are in approximate equilibrium it is clear that intra-uterine conditions have practically reached an impasse. Further growth of the embryos would demand more oxygen, but that oxygen could not be supplied from the available supply except at the expense of a grave decline in the already small pressure at which the gas is supplied to the embryos. This pressure is already of the same order as, or below, that in the alveolar air at the top of Everest.

The rôle of the uterine veins.

We are now in a better position to discuss, though perhaps not to answer, the question which formed the original *motif* of the present paper. To what extent may the uterine vessels be regarded as a blood depot?

The discussion really concerns only the great development of veins.

Is this vast bed necessary for the transport of the quantity of blood which passes along it? The answer must surely be "no." For this answer three reasons may be given:

(1) The following argument may be applied: (a) Approximately the same quantity of oxygen must pass from the placenta to the foetuses as from the maternal blood to the placenta. (b) The maternal blood being more than half reduced in the process, it may be assumed that at least as much blood passes along the umbilical veins as the uterine veins: yet, (c) The umbilical veins are delicate structures and, as compared with the uterine veins in the rabbit, are of almost negligible size.

(2) Each uterine vein at the end of pregnancy appears larger than the iliac vein into which it flows—yet there seems to be little difference in size between the portion of the iliac vein above the uterine vein and that below it.

(3) Veins no larger in other parts of the body carry much greater quantities of blood.

When we speak of a depot, however, there is the suggestion that the blood is stored for some specific purpose. For what purpose could blood be stored in the uterine vessels, to be liberated suddenly at parturition? The obvious suggestion is for the supply of mammary tissue much

hypertrophied during the active process of lactation. We do not know the quantity of blood in the mammary glands, but a few rather crude measurements suggest that they contain much less than 30 c.c. This, however, must form the subject of future enquiry.

Another useful function of a store of blood would be insurance in case of hæmorrhage during parturition. Meanwhile it is scarcely possible to accept the idea of the great veins in the broad ligament being a blood depot without considering some possible alternative functions. Of these the most probable appears to be protection against a too high venous pressure. This may be considered in three connections:

(1) The copious anastomoses which exist go far to prevent pressure on any one vein from producing stasis in the corresponding placenta.

(2) In several experiments we have been much impressed with the tendency of even moderate pressure on the uterine vein to produce intra-uterine hæmorrhage. On two occasions, not in this but in a former series of experiments, when there was some slight bleeding in veins near the main uterine vein and in which pressure was used very cautiously to stop that bleeding, considerable hæmorrhage was found in the uterus of the same side and not of the opposite side.

(3) It has already been noted that at birth the pressure at the mouth of the uterine vein oscillates within wide limits. It is possible that with the blood being driven from the smaller vessels of the uterus in a more or less rhythmic manner, this large venous bed may act as a sort of shock absorber, filling and emptying according to the rate of flow from the uterus itself and insuring against so great a venous pressure as to produce copious hæmorrhage.

SUMMARY AND CONCLUSIONS.

1. The blood becomes appreciably concentrated in its passage through the uterus.

2. The blood flow through the uterus increases in volume in proportion to the increase in the vascular bed and anticipates the growth of foetal tissue.

3. The blood flow in the rabbit's uterus attains a maximum of about 30 c.c. per minute.

4. The oxygen used by the pregnant uterus and its contents is less than 0.1 c.c. per minute up to the eighteenth day; within the next two days it undergoes a tenfold increase, corresponding to foetal growth. After that time the oxygen used increases much more slowly and at a rate proportionately less than the growth of the embryos.

5. The oxygen saturation of the venous blood which is from 80 to 90 p.c. on the fourteenth day falls to 25 to 45 p.c. by the end of pregnancy.

6. The function of the extensive venous reservoir in the broad ligament is discussed (*a*) in relation to its possible function as a blood depot, and (*b*) as an insurance against stasis or intra-uterine hæmorrhage caused by local pressure.

Our thanks are due to the Royal Society for a grant which defrayed a part of the expense of the above research.

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THE COMPONENTS OF THE RETINAL ACTION
POTENTIAL IN MAMMALS AND THEIR
RELATION TO THE DISCHARGE IN
THE OPTIC NERVE.

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Part I. Isolation of components in the retinal action potential
of the dark-adapted decerebrate preparation.

OUR knowledge of the retinal action currents, discovered by the Swedish physiologist Holmgren [1882] in 1865, has proceeded hand in hand with the development in electrophysiology in general. The history of this striking progress in electrical recording is briefly summarized in the literature relating to retinal action currents. Since Gotch [1903], working in this laboratory, with the aid of the sufficiently fast capillary electrometer, obtained the first curves embodying all the features of the process, and since v. Brücke and Garten [1907] and Piper [1911] in extensive series with the string galvanometer had shown the responses to light to be fundamentally alike for various vertebrate eyes, the main features of the retinal action currents have been common knowledge to all physiologists. Valve amplification was used at an early stage for the investigation of retinal action potentials by Chaffee, Bovie and Hampson [1923]. Unfortunately they used excised opened bulbs, although the method was particularly well suited for the study of intact animals, a feat attempted as early as 1876 by Dewar and McKendrick [Dewar, 1876]. With their slow Thomson galvanometer the latter authors even succeeded in obtaining responses from the human eye, but it remained for Hartline [1925] to prove by systematic comparisons with the string galvanometer that the deflections obtained from intact animals were identical with those given by the bulbs. Hartline also recorded some fairly good retinal action currents from the human eye.

The retinal action currents have generally been held to be composite effects. In view of the complex structure of the retina and the equally complex appearance of the potential change accompanying stimulation by light, interference phenomena between potentials differing in sign,

¹ Fellow of the Rockefeller Foundation.

strength and time relations would certainly offer a reasonable explanation of the effect in terms of simpler components. Several such solutions have been propounded [see *e.g.* Kohlrausch's review, 1931], the best known being those of Einthoven and Jolly [1908] and of Piper [1911]. Evidently it is theoretically possible to resolve a complex curve in an infinite number of ways. And, though a many-sided experimental experience may make certain solutions more probable than others, yet a final decision can only be reached when the composite curve has been split into components by biological means. Such an attempt forms the subject of this paper.

The work has been based on the assumption that an organ like the retina where cells have become differentiated for specific purposes may show selective sensitivity or selective resistance to certain agents. It then becomes of paramount importance to find a preparation sufficiently stable and yet sufficiently sensitive to serve for the analysis. Frogs were tried but soon discarded in favour of the Sherrington decerebrate cat preparation [cf. Hartline, 1925]. This proved very satisfactory, provided that no operations were carried out around the bulb. In the best animals the first positive deflection, the *b*-wave, remained constant within 4–5 p.c. for several hours. The secondary rise varied more. Some thirty animals were used and the number of photographed responses approached 800.

ANIMAL TECHNIQUE.

Cats were decerebrated under deep anaesthesia by a "backward" section generally carried down to the base of the skull. A dilated pupil was thus obtained. In a few cases atropine had to be given to immobilize the iris. This did not seem to influence the action potentials. The modified decerebration technique, described by Bazzett and Penfield [1922] and used by Hartline [1925] in order to prevent interference with anastomotic connections at the *basis cranii*, was not found (in ten preparations) to possess any advantages over the ordinary clean section. The nictitating membrane was removed and the lids tied apart. The carotid of the side to be recorded from was temporarily occluded during the operation.

The preparation was placed in a shielded and earthed box, the inside of which was painted black. The head of the animal was fixed in a specially constructed clamp and adjusted with one eye towards the opening of a tube leading to the stimulus and entering the box. This was placed on a heated table of the type used in this laboratory: 2 to 3 hours were allowed to elapse before any records were taken. The box had then been

closed for $1\frac{1}{2}$ hours or more, except for the moment when the corneal electrode was applied. This was done about half an hour before the first records were taken. Faint light was used during this procedure.

Apparatus.

Contact with the cornea and the decerebration wound was made through cotton wicks leading to U-tubes filled with Ringer solution into which dipped silver-silverchloride wires. The leads were taken to the input of a directly coupled amplifier containing two valves coupled against one another in what is generally known as a "push-pull" arrangement. A small permanent magnet string galvanometer (Edelmann's small model) was placed in a bridge between the anode circuits and balanced to zero by potentiometers in the grid circuits. This coupling scheme, for which I am indebted to Dr H. K. Hartline of the Johnson Foundation, University of Pennsylvania, gives a circuit practically free from drift. A different type of directly coupled amplifier was used by Chaffee, Bovie and Hampson [1923].

The valves were 41 M.X.P. Cossor with indirectly heated filament, high mutual conductance, and low amplification factor. The actual amplification is only about five times. The use of valves was dictated, not so much by any necessity of amplifying the retinal currents, as by the advantage of having a potentially worked device such as the grid of a valve. This renders the resistance in animal and electrode negligible. Valves also make it possible to use the fairly insensitive permanent magnet string galvanometer. Further advantages are the cheapness of this galvanometer and the fact that the magnet and the case of the string remain at a practically constant temperature during the experiment. There is thus no need for continuous adjustment of string tension followed by calibration. As a matter of fact the calibration current was turned on only once or twice an hour, depending on the nature of the work. A disadvantage of amplification in this particular type of work is that not only action potentials derived from the retina but also those caused by movements in the eye muscles, lids, etc., are amplified. However, it can be seen from the records published below that good preparations are fairly stable.

The string was generally kept aperiodic or just periodic with a rising time not exceeding 20σ . The fastest *b*-waves recorded rose in about 50σ to their maximal value. The deflection to 1 mv. was rarely over 40 mm. through animal and amplifier. A platinum string 2 micra thick and with a resistance of 3100Ω was used throughout the work.

Since the permanent magnet string galvanometer has not been used very much as a measuring instrument on account of its low sensitivity, it may be useful to mention the disadvantages connected with this apparatus. The adjustments for string tension and focus are crude. The microscope and the magnet are not well balanced on the small upright to which they are attached. Hence the apparatus is very sensitive to mechanical disturbances. The string cannot be precisely centred in the magnetic field and the proportionality range is low. The latter fact is of little concern when the string can be kept slack. The range of proportionality was tested and found to be satisfactory within the limits of deflection used in this work, where not only was the string fairly slack but the optical magnification was considerable (approximately 600 times).

The stimulus was supplied by a Primus projection lamp (1000 c.p.) with reflector. The light beam was led through a system of lenses and screens and focussed to a narrow opening in a diaphragm. An adjacent minute slit let part of the light through a series of prisms to the camera. A Compur photographic shutter at the focussing point interrupted stimulus- and signal-beam simultaneously. The light reached the box through a system of concentric tubes of which the innermost possessed a ground glass disc with an area of 1661 sq. mm. on which an image was formed.

Though several intensities were used the typical effects are well illustrated by the highest intensity, 14 millilambert, and a 100 times lower intensity. The high intensity will be referred to in the text as I, other intensities as fractions of I. The intensity was varied by means of Wratten filters and was measured by a Lummer-Brodhun contrast photometer and a lamp standardized by the National Physical Laboratory. These were obtained when the apparatus had been used for nearly 5 months, so that the intensities given do not take into account the ageing of the lamp.

The cat's retina.

Some eyes were fixed in "Susa" and stained in Heidenhain's iron-haematoxylin. In accordance with older observations [cf. *e.g.* Chiewitz, 1889] the retina was found to contain very few cones. Also the convergence of receptors towards ganglion cells was found to be considerable, between 30-60 cells in the external nuclear layer per ganglion cell, in sections from various parts of the retina. In the human periphery the corresponding figures vary between 20-80, the latter value in the outermost parts of the retina [Chiewitz].

The normal action potential.

Some typical specimens are shown in Fig. 1. *A*, *B* and *D* are at the highest intensity, *C* is at $I/100$, *E* is at $I/10$. *A* is taken at a slower speed with the stimulus lasting 11 sec. and 5 sec. cut out from the film. The secondary rise is the largest noted in these experiments. A suitable terminology for designating the various phases of the retinal action potential was suggested by Einthoven and Jolly [1908]. Thus the initial fast negativity is called *a*, the first positive rise *b*, the slow secondary rise *c*. The off-effect (Gotch's term) was lettered *a'* by Einthoven and Jolly. Below, *d* is used [Day, 1915].

The three high-intensity curves show typical variations in the relative size of the waves. *B*, again, is typical of the large majority of the high-intensity responses. The *a*-wave is only present with high intensities and with large areas. The same holds for the off-effect or *d*-wave, which is not always present and is hardly ever more than a retardation in the falling part of the *c*-wave [cf. similar observations by Piper, 1905, 1911; Kohlrausch, 1918]. The very simple character of the response at low intensities should be noted; likewise the fact that occasionally, as in *D*, the base line may be re-attained for a moment after the *b*-wave at high intensities. The *a*-wave is not an abnormal phenomenon due to excessively high intensities of stimulation. It is a perfectly normal phenomenon and can, for instance, be seen in the human response curves published by Hartline [1925]. Though 14 ml. as a maximal intensity is not very high for the light-adapted human eye, it is a strong stimulus for a dark-adapted nocturnal animal with a wide-open pupil.

The effect of area.

This question will be treated in greater detail in Part II, where some measurements of the latent period of the action potential will be published. Here it is only necessary to emphasize the fact that a diminution in area affects the action potential just as a diminution in intensity [cf. Kohlrausch's review, 1931]. Thus the response for small areas of about $1-2^\circ$ of visual angle differs from that for areas over about 5° in lacking secondary rise, the *a*-wave and the *d*-wave. They resemble the low-intensity curve *C* of Fig. 1.

Fröhlich [1928] and Fröhlich, Hirschberg and Monjé [1928] contend that the ordinary complicated responses depend upon the fact that the leads are not localized to the point of stimulation. Kohlrausch [1918, 1931] has convincingly shown that this hypothesis is untenable.

It may be added that the alleged proofs published by Fröhlich, Hirschberg and Monjé refer to experiments in which the area stimulated was not only kept just in front of the electrode but was also diminished in order to obtain strict localization. Inasmuch as the responses then became simplified, this result might just as well be ascribed to the diminution in area as to the fact that the lead was at the point of stimulation. In my experiments the corresponding lead was taken from the decerebration

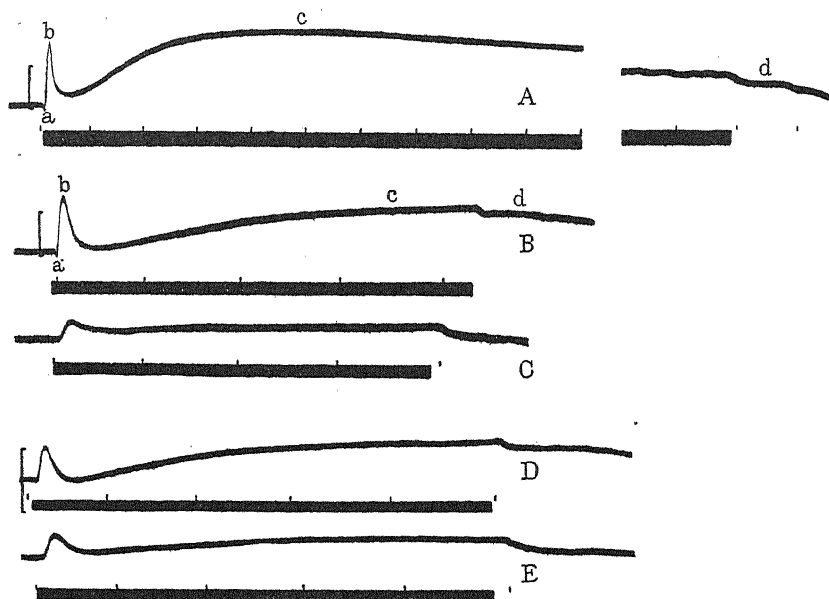


Fig. 1. *A*, intensity I; 5 sec. cut out. *B*, intensity I. *C*, intensity I/100. Standard area at 70 mm. in this and all following records except *D* and *E*, which are taken with the same area at 370 mm. at respectively I, and I/10. The potential developed in *D* and *E* is unusually low even for this retinal area, though normal in appearance. The deflection to $\frac{1}{2}$ mv. is marked on one of each of the records of a series with the same animal, in this and in all illustrations following. Time is marked in all records in 0.5 sec. In addition time is marked by the shadow of the Rayleigh wheel giving 100 and 20 σ when visible in reproductions of original curves.

wound on the skull and yet the responses became simplified by merely diminishing the area. No localization of the electrode is therefore necessary. The results to be described below put Fröhlich's theory definitely out of court. But the manner of leading off naturally is of some importance, particularly with a large excised eye as used by Fröhlich in some cases (Cephalopod), where probably his explanation holds.

Removal of c-wave.

The *c*-wave varies a great deal in height from animal to animal. It is extremely slow as is well shown by Fig. 1, curve *A*. It generally reaches its maximum in about 2 sec. or slightly less, and may last for several seconds after stimulation.

In order to remove it ether was chosen as a differentiating agent and was found to be very satisfactory. It was administered by way of inhalation. Fig. 2 (Plate I) shows three ether experiments in which the high-intensity stimulus was employed. In the upper pair of curves it can be seen that narcotization has removed the rising component of the *c*-wave in 14 min., and that the fast components are unaltered. The string remains at a practically steady low potential. In the next pair of records, of which the upper one again is the control, the picture after 14 min. of narcotization is similar, but the base line has shifted a little towards the end of stimulation. It is important to note that in this record the off-effect is enhanced by narcotization. This, in fact, is very often found. The third pair of records shows a case where 9 min. of anaesthesia has left the fast components unchanged, but greatly diminished the secondary rise. Whereas in some animals the rise may be completely removed by narcotization without any measurable effect on the fast initial components, there are certainly cases where part of the secondary rise is left at a stage of anaesthesia when a diminution of the *b*-wave is already noticeable.

It can be shown that the remaining part of the *c*-wave, in cases where something does remain, is not identical with the component that has been removed. The procedure is to analyse the effect of narcotization at two intensities. In Fig. 3 the upper pair of responses shows the control curve and the potential elicited by the stimulus I after 29 min. of narcotization. The lower pair shows the corresponding curves at 1/100. The ether effect on the responses is very different. Whereas the *c*-wave in the high-intensity record has diminished by 54 p.c., it is only diminished by about 8 p.c. at the low intensity. High-intensity records were taken before (the one published) and 2 min. after the low-intensity record, and showed that the lack of effect with the lower intensity did not depend upon insufficient narcotization or recovery from the anaesthesia. The explanation is clearly that the rising component, which is such a marked feature of the high-intensity response, is minimal at the 100 times lower intensity. Other experiments showed it to be absent or below the instrumental threshold at still lower intensities. The low-intensity curves were unchanged at a stage of anaesthesia showing high-intensity responses

affected as in Fig. 2. In this manner, then, it is possible to determine the time course and magnitude of the slow secondary rise, and, in fact, to isolate it by subtraction.

It is evident that the purely descriptive term, *c*-wave, has a double sense in terms of components. The *c*-wave is not homogeneous at high intensities. With certain animals nearly the whole of the secondary positive potential can be removed with ether at high intensities. It is then not identical with the corresponding component at low intensities which is unaffected. The component that is so easily removed by narcoti-

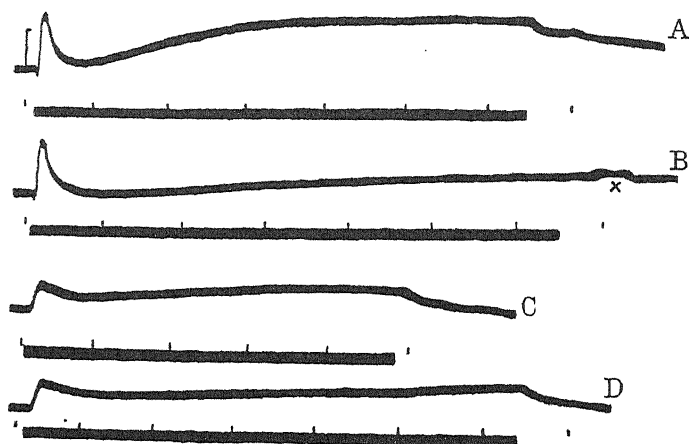


Fig. 3. Full description in text. Cross marks artefact following slightly increased off-effect.

zation will henceforth be termed the first process, P I. At times most of it disappears in 5 min. when there is still reflex activity left in the animal. Sometimes P I is more resistant.

Final effects of narcotization.

Further narcotization begins to affect the *b*-wave, which diminishes in height. When this happens the latent period, so far constant for a given intensity, begins to lengthen, and generally the rate of rise of the *b*-wave also becomes slower. This effect can be noted at all intensities. If the original intensity has been high the positive slow remainder after removal of P I often becomes replaced by a slow negative deflection. The final result is always that the whole response becomes negative, provided the intensity has been high enough to elicit a negative effect. A large number of such negative responses have been published by previous

workers [see below and Kohlrausch, 1931]. The early investigators, whose technique involved elaborate dissection, saw practically nothing but the apparently very resistant negative responses with mammalian eyes [see *e.g.* Holmgren, 1880; Dewar and McKendrick, 1873]. To the work of Kühne and Steiner [1881] we owe the knowledge that the response to white light should be chiefly positive [cf. also Dewar, 1876].

The negative response is influenced by ether just as is the *b*-wave. During continued narcotization its latency begins to lengthen, the potential diminishes and the rate of fall becomes slower. These effects can often be noticed before the *b*-wave has disappeared. The most

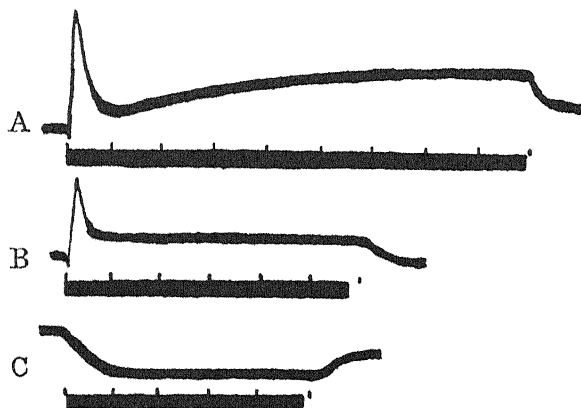


Fig. 5. Progressive effect of anaesthesia recorded at I/10. *A* is control before narcotization. *B*, after 21 min. of anaesthesia. *C*, after 31 min. of ether. Slack string, *b*-wave of *A* giving 0.417 mv.

marked alteration, however, found already at an early stage of anaesthesia, is that the return to the base line after cessation of the stimulus becomes sluggish. Fig. 4 (Plate I) shows part of a typical negative response of this type. All this makes it extremely difficult to intercept with the camera a negative wave at a moment when it can be proved to be uninfluenced by ether. An early negative wave was photographed in the experiment shown in Fig. 5. The intensity is I/10, the uppermost curve showing the normal response. The next curve shows a stage of anaesthesia where the positive remainder after removal of P I is already affected. The final negative wave in this case falls rapidly and also rises fairly rapidly at the cessation of the stimulus. It is larger than most of the negative waves seen in these experiments.

If the narcotic be removed at this stage of final negativity, and, if necessary, the animal be given some artificial respiration, complete recovery is generally possible. All the effects are thus reversible. Often the secondary rise is enhanced some time after recovery has taken place. Likewise when ether is first given there may be a stage of very short duration when P I is temporarily increased. Similar effects are also at times observed with the *b*-wave.

The final stage in progressive narcotization is complete disappearance of the response. This stage may mark an irreversible change. It has not been possible to revive the retina once it has ceased to respond.

By comparing ether effects at different intensities of stimulation it is again possible to separate into components the curve remaining after removal of P I. Thus, at low intensities, removal of the total positive remainder shows that this was practically the only component present, since no effect whatsoever or a minute negative response is all that is left at a stage of anæsthesia when there is a large negative deflection at high intensities. The positive remainder after removal of P I reacts uniformly and simultaneously to ether at all intensities of stimulation and will, therefore, be designated by the term P II. The negative wave will be termed P III. All three processes, P I, P II and P III, react in a similar manner to ether, though a certain amount of selective resistance to the narcotic makes it possible to remove them in three characteristic steps. The normal action potential to weak stimuli consists of an almost pure P II. This makes the cat a very suitable animal for an analysis of the kind attempted in this paper, for any effect on P II can always be checked by using a low-intensity stimulus.

Removal of P II.

The fact that it is difficult to intercept a negative wave at a stage of anæsthesia when its latency can be proved to be unaltered may depend upon deficient selectivity of the narcotic with regard to P II and P III. It is, therefore, necessary to try some other means of removing P II from a high-intensity curve in order to determine the shape and latency of the negative wave, particularly with reference to the initial brief negative deflection. It is also necessary to test the analysis obtained from the ether results by some other method.

A case in point is illustrated by Fig. 6 (Plate II). *A* is a normal response to I. Then the carotid is occluded for 2 min. and *B* is obtained. The *a*-wave is slightly accentuated, the *b*-wave has become very small. It is now both followed and preceded by a negative deflection. P I is enhanced by the

asphyxia. The off-effect is definitely larger having been only a retardation in the post-stimulatory fall in the previous normal response. The animal has moved just before the light goes off. Troublesome movements generally occur when the carotid is occluded. The off-effect represents, however, a real increase, as has been confirmed in several similar cases, and may be seen in curve *H* in the same figure. *C* shows the initial part of the response to the same stimulus 2 min. later. Then the carotid is released, and 14 min. later a new record *D* is taken showing full recovery and persistence of the enhanced P I. The carotid is finally occluded once more, and a record *E* is taken 5 min. afterwards. Apparently then some collateral compensation has taken place, since the record is normal in form but shows diminished deflections. *F* shows a normal response to the same intensity in another experiment. Then the animal was curarized and *G* was taken showing one further stage in the process begun above by arterial occlusion. Curare may affect the *b*-wave to some extent—I have seen it do so—but the curve may still be of the normal type [Kohlrausch, 1918]; there was, however, a serious leakage in the tube leading to the pump of the respiration apparatus, and this must be the explanation of the almost complete removal of the *b*-wave. The *b*-wave is only a retardation in the negative deflection initiated by the *a*-wave, and the initial negativity *a* runs almost directly on into the large negative P III. The latency is constant, about 20σ in both records.

Curare was used to induce asphyxia without troublesome movements in the animal. But it was deemed unnecessary to continue these experiments since the question appears to have been settled by Kohlrausch [1918], who used a curarized rabbit (rod-eye). The results obtained by arterial occlusion may just as well be brought about by stopping the respiration in a curarized rabbit. Kohlrausch accidentally noted a response of the type shown in *B*, *G* and *H*, and then found that the respiration apparatus did not function properly. Adjustment of the artificial breathing brought the response back to normal again. His deficient curve shows no *b*-wave, an enhanced secondary rise and an increased off-effect. It looks, in fact, precisely like curve *H* in Fig. 6 (Plate II)¹.

The deficient response *H* was the result of an unsuccessful operation. Probably the carotid was occluded too long, since this in some animals leads to irreversible disappearance of *b*. In this case the animal gave the same type of response for some time. In other similar cases there either appeared later a small *b*-wave or the response diminished in amplitude.

¹ In later experiments, some of which are mentioned in Part II, asphyxia after occlusion of the carotid sometimes completely removed P II.

fairly rapidly. When in such cases ether was given, removal of the secondary rise left a pure negative remainder. In order to find out by some other means whether the second phase of P II, which in high-intensity curves is covered by P I, is lacking in responses of this type, the 100 times lower intensity was used. Curve *I* gives the response to this stimulus, adjusted to the same light signal as *H*. Of the considerable negative practically nothing is left at this intensity, and of the large secondary rise there is but a fraction. This wave has a very long latency just as P I. The fraction left also corresponds to the amount of P I that the ether analysis (cf. Fig. 3) showed may be present at this intensity. P II at this intensity is much larger and quite different as clearly shown by Fig. 1, curve *C*. The negative P III also behaves with respect to intensity as was to be predicted by the results obtained with ether. Evidently then P II in addition to the *b*-wave contains a second positive phase, which is practically pure at low intensities. At these intensities P I and P III are small or absent. The response *H* is a combination of P I and P III.

Components in relation to stimulus.

By using ether it was possible to obtain P II alone at low intensities, P III alone at high intensities, and also to produce the response P II + P III. By interfering with the oxygen supply it was possible to produce the response P I + P III. It has not been possible to obtain P I alone. Thus it follows that P II and P III are very directly related to the stimulus. It does not seem probable that the one elicits the other. As to P I, it may, of course, merely be a matter of finding the right procedure in order to obtain it alone, but so far it seems as if P I required P III. It does not require P II to judge from the large P I in the response P I + P III.

P III in relation to a- and d-waves.

The evidence so far obtained allows certain conclusions to be drawn regarding the place of the *a*-wave and the off-effect in the analysis of the composite effect. The ether experiments (cf. Figs. 2, 3) often show an enhanced off-effect after removal of P I. Thus P I is not necessary for the off-effect to appear. On the other hand, removal of P II at high intensities regularly increases the off-effect as confirmed by Kohlrausch's similar findings. Therefore P II cannot cause the *d*-wave. This also follows from the low-intensity curves which contain a large P II and practically nothing else. They never show an off-effect. Thus the component necessary for the *d*-wave to appear is the negative P III and, in addition, either P I or P II to serve as a background against which the return to zero of P III

at the end of stimulation can set itself off. For the alternative explanation that P III actually rises above the base line at the end of stimulation there is no evidence. The purer the negative waves the more definitely are they monophasic. Since P I and P II drop at the cessation of the stimulus, it follows that the absence of either counteracting fall must make the off-effect caused by the return to zero of P III more marked. P II drops faster than P I. Therefore, the removal of P II must cause particularly large off-effects which actually is the case. Kohlrausch [1918], using Piper's analysis, gives the same explanation of the large off-effects after removal of P II by interfering with the artificial respiration. The experiments reported above settle the fact that the off-effect depends upon the behaviour of P III.

The negative waves obtained after ether in general possessed too long latencies to have started before the positive P II, though, on the other hand, the effect of the narcotic upon the latency made it probable that, if a negative could have been obtained at a sufficiently early stage of anaesthesia, it might well have started before the positive wave. The experiments on removal of P II by interference with the blood supply gave the additional information wanted. A series of records was obtained showing the initial *a*-wave gradually running on into the large negative P III. This time the latency was not altered, but remained at about 20σ , the value of the latent period of the *a*-wave. Not only, therefore, is it unnecessary, but it is also unreasonable to assume a separate twitch-like initial *a*-wave when all the evidence shows it to be closely related to the large negative P III. Thus it is influenced by area and intensity just as P III, and is lacking in the pure P II responses.

Previous analyses will be mentioned below, but in this connection some of the facts relating to negative waves deserve to be pointed out separately. Thus Piper [1911], confirmed by Kohlrausch [1918], found that vertebrates (fishes, amphibians, reptiles) which had large *a*-waves also had good off-effects, whereas those (certain mammals as rabbits, cats, dogs) which gave small and inconstant *a*-waves also had small or inconstant off-effects. These two, the *a*- and the *d*-wave, must therefore be ascribed to the same process, which above was shown to be P III. The same conclusion was reached by Einthoven and Jolly [1908]. Waller [1909], confirmed by Jolly [1909], obtained fast monophasic negative waves by giving frog's eyes massage. Waller found these negatives to possess a shorter latency than the positive deflections. The return to zero was also fast. These facts he incorporated in his analysis [cf. also Einthoven and Jolly, Piper, Kohlrausch].

Nikiforowsky [1912] obtained negative waves by cooling frog's eyes. He, as later Tirala [1917], found that the *d*-wave cannot be due to the same process that causes the *b*-wave, above demonstrated to be P II.

The analysis of the composite effect.

The analysis of the retinal action potential in the dark-adapted cat's eye may now be given with a fair degree of confidence. There are three processes numbered in the order of their disappearance during progressive anaesthesia. Their relative contributions to the composite effect varies with area and intensity as set forth above. Since an increase in area affects the response just as an increase in intensity, it is only necessary to solve for two intensities. Besides, the effect of area, owing to effects of

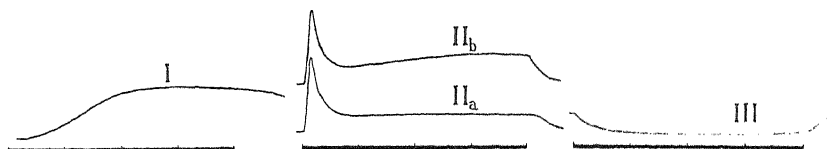


Fig. 7. Components of retinal action potential to full I for stimulus lasting 2 sec. Two alternatives are given for P II. For P II, as in II *a*, P III should be constructed rising as II *b* to fit cases in which, after removal of P I by ether, the composite potential still shows a secondary rise.

shunting by the tissues, cannot be as reliable an index of what takes place as the effect of intensity. The low-intensity response is simpler and the components are therefore only given together with the composite curve in Fig. 8. The high-intensity components are also given separately in Fig. 7.

Since the high-intensity second process, P II, cannot be obtained separately at this intensity, it is evident that the rise which sometimes is observed after removal of P I may be solved from a rise in P III or a rise in P II itself. Isolated third processes have sometimes in the course of this work been found to rise slightly during continued illumination. If this rise in some animals were large enough it could account for the rise observed after removal of P I. That would necessitate a P II of the type *a* in Fig. 7. The same type of P II would be obtained if for some reason in certain animals P I were unusually resistant, though the evidence with low-intensity second processes does not support this view. The same experimental response, P II + P III, after removal of P I could also be obtained if P II itself rose slightly as shown by curve *b* in Fig. 7. The latter alternative finds some support in evidence to be presented in Part II.

There the components will be evaluated in terms of processes in the optic nerve. The composite curve of Fig. 8 is constructed on the basis of a P II of the type *b*. The figure summarizes the results of the analysis carried out above and needs no further comment. It may, of course, be constructed to give a larger off-effect after removal of P I.

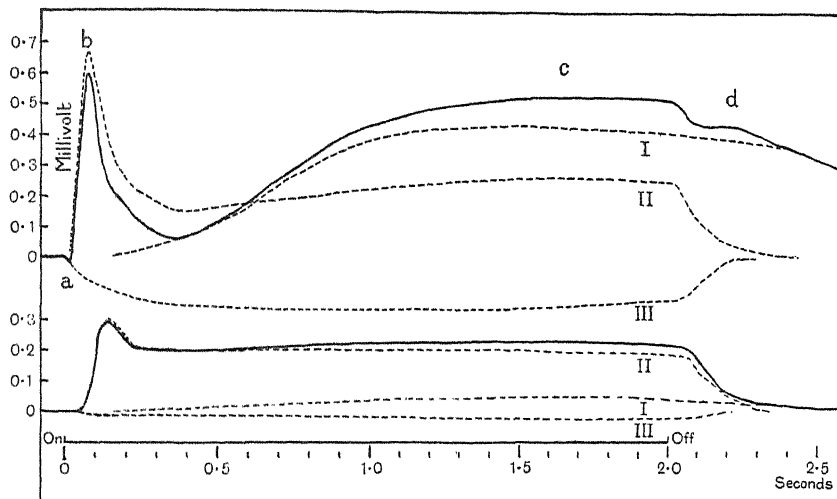


Fig. 8. Analysis of composite retinal action potential at two intensities, 14 ml, and 0.14 ml, and area of 1661 sq. mm. viewed at a distance of 70 mm. Components: broken lines. Composite curve drawn in full. The *a*-wave is broadened slightly out of scale to show its derivation more clearly.

Previous analyses.

In view of the fact that the retinal action potential for various vertebrate eyes contains the same phases and that histological evidence shows all such retinae to be built on a similar plan, the analysis must be general in principle. It therefore becomes particularly interesting to compare it with the two most important analyses, *i.e.* those by Einthoven and Jolly [1908] and by Piper [1911]. Behind these two attempts there is not only a many-sided experimental experience [Piper], but also systematic analysing of response curves obtained under various experimental conditions [Einthoven and Jolly], [Kohlrausch, 1918]. The slow instruments used by the early workers in this field, often in connection with severe operations, made delicate work impossible. A notable exception is the very interesting contribution by Kühne and Steiner [1881].

Einthoven and Jolly recognized the three processes, or "substances" as they call them. P I is quite correct, P II lacks the slow second phase showing that they did not realize that the *c*-wave is not homogeneous. P III gives the negative as a twitch below the base line and the off-effect as a positive deflection. The intervening negative phase is lacking. It is important to note that they found that the *a*-wave and the *d*-wave had to be due to the same component. Piper improved upon this analysis by introducing the intervening negative phase of P III, and by not allowing it to end in a positive process. In fact, his negative wave is strictly monophasic and the off-effect as well as the *a*-wave are solved by precisely the same interference construction as above in Fig. 8. This negative wave had in the meantime been found by Waller [1909], who corrected Einthoven and Jolly's solution in this respect. He resolved *d* and what he believed to be *a*, as did Piper later, but made the mistake of using only two components, a positive and a negative. He also believed the fast *a*-wave to be abnormal. Piper further realized that the *c*-wave is not homogeneous, but contains P I and the second phase of P II. P I is identical with the corresponding "substance" of Einthoven and Jolly. P II differs from the analysis given above in that it rises rapidly to a maximum which then is retained during continued stimulation. It is a low-intensity P II added to high-intensity first and third processes. This P II was based on his experiences with the Cephalopod eye [1911] which, of course, is analogous only, not homologous with the vertebrate retina. It is possible that in some animals the fall of the *b*-wave is mainly due to P III rather than to a decline in P II. In the cat at high intensities this certainly is not the case. Evidence to be presented in Part II makes it somewhat doubtful whether it can ever occur at such intensities. Still, Piper's analysis is essentially correct, though largely hypothetical, and the general interest taken in Einthoven and Jolly's and Piper's combined efforts to solve the retinal action potential has not only been fully justified by the work presented in this paper, but also by Kohlrausch's experiments with various colours. He there found that the short wave-lengths produced very small second processes followed and preceded by negative deflections, resembling, in fact, curve *B* of Fig. 6. The long wave-lengths, on the other hand, had a greater effect upon P II. Both lights together gave summed curves, which not only corresponded well with the theoretical composite curves but could also be explained on Piper's solution. Most important was the finding that partly negative deflections can be obtained in, for instance, a cone-eye like the pigeon's by stimulating with short wave-lengths. This rules out the supposition that a

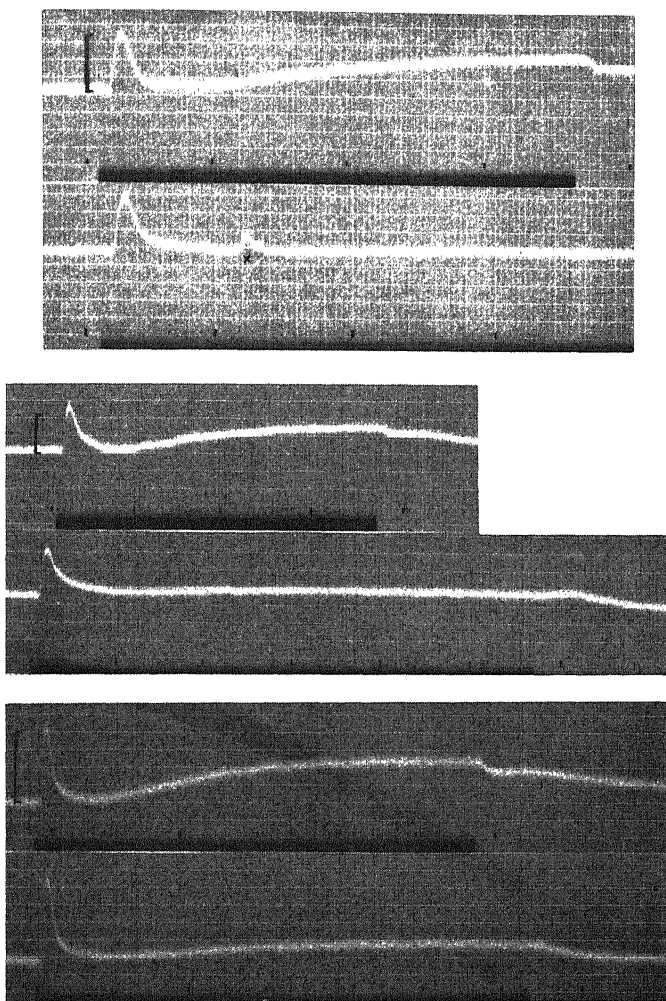


Fig. 2. Three deflections to full I during initial stage of light anaesthesia together with controls taken before narcotization. Full description in text. Artefact marked by cross is probably movement in animal's eye.

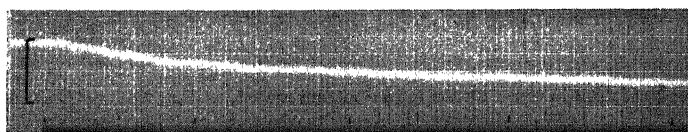


Fig. 4. Negative deflection to full I obtained after prolonged narcotization.

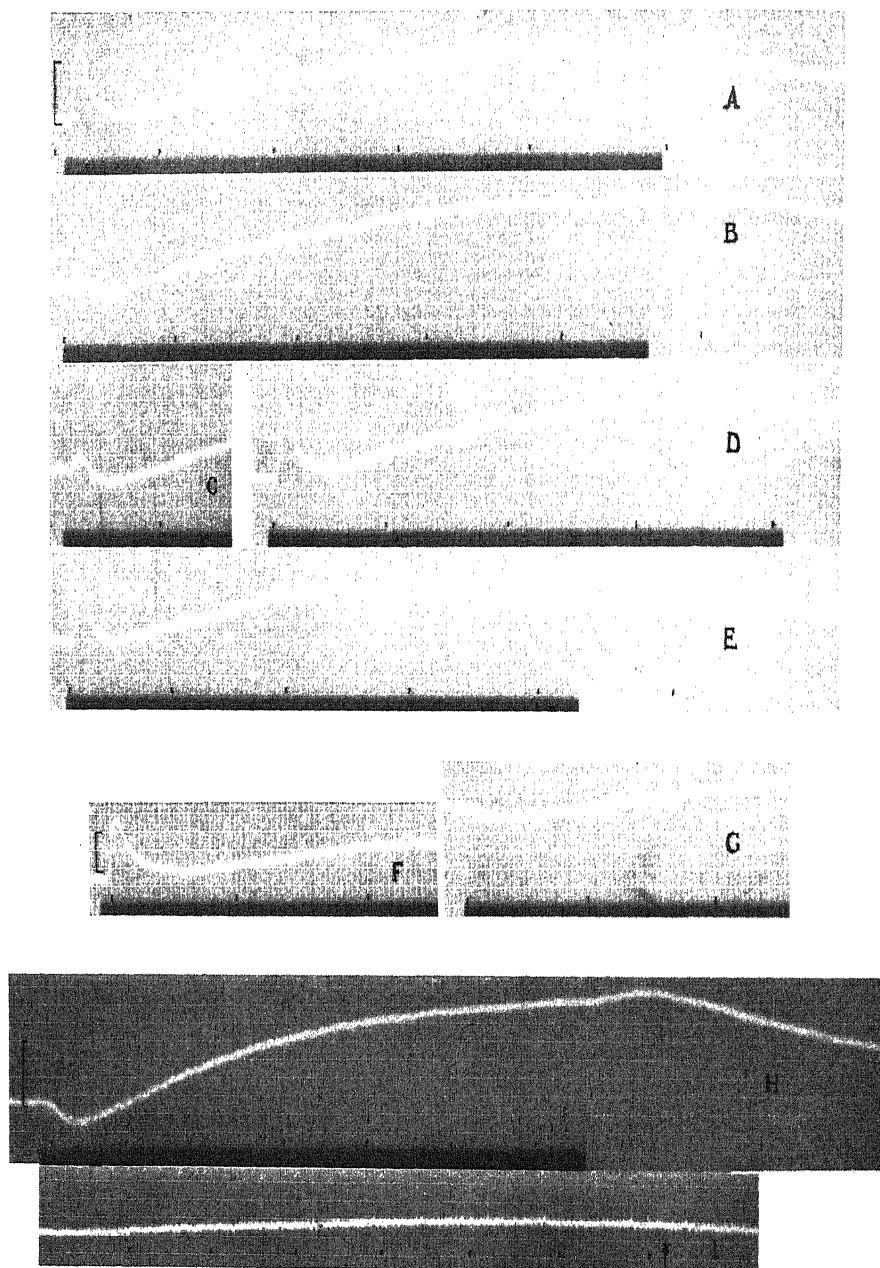


Fig. 6. Full description in text. Deflection in bottom record, to save space, adjusted to light signal of foregoing record. Arrow marks end of stimulation.

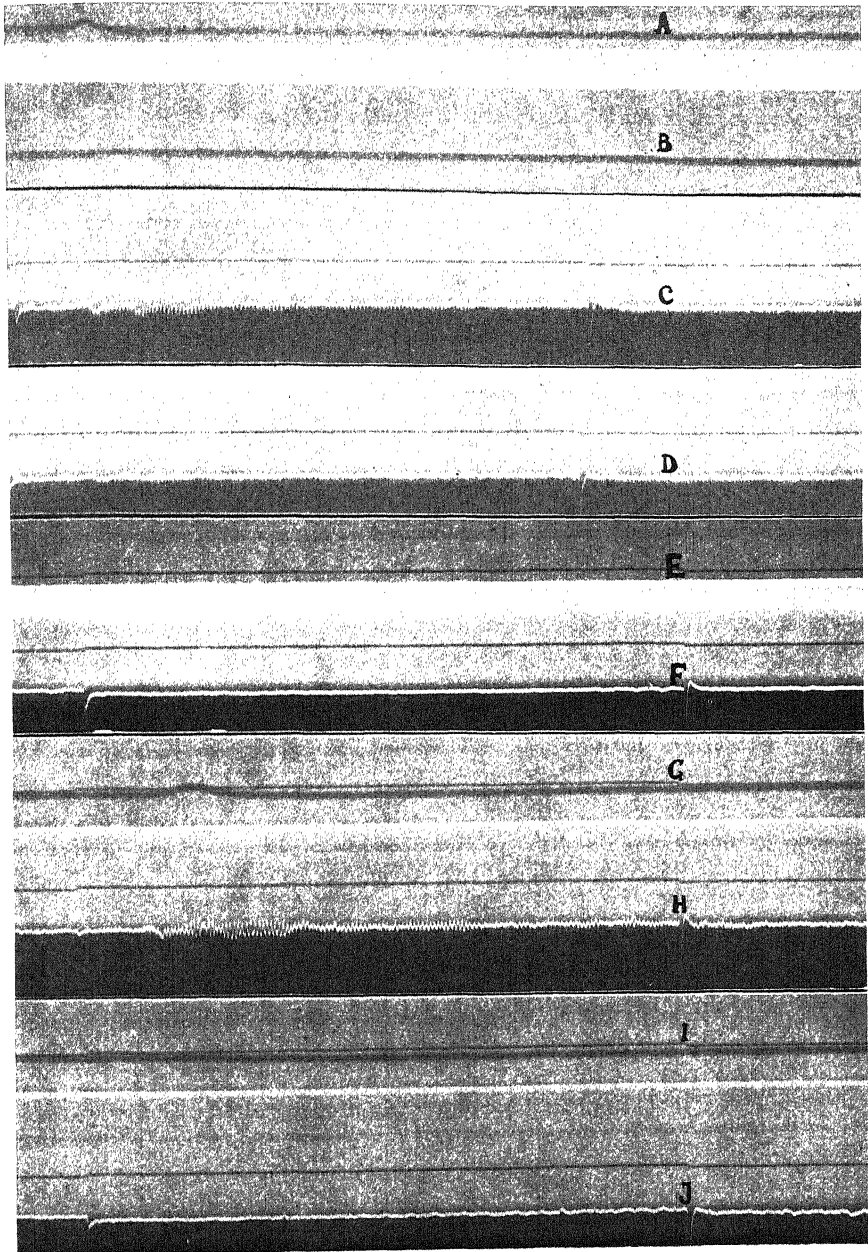


Fig. 9. String galvanometer records of retinal response and oscillograph records taken from the optic nerve. Time: tuning-fork, 100 per sec. Signal is seen causing escape at on and off of light, picked up by oscillograph. Explanation in text.

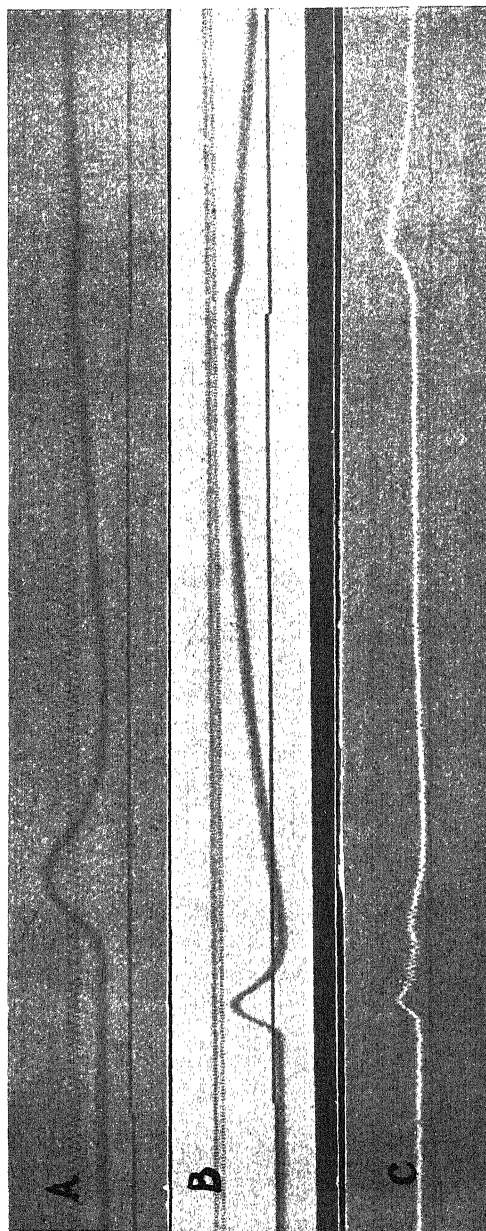


Fig. 10. *A* and *B*: string galvanometer records of retinal action potential. In *A* stimulus lasts about 1.3 sec., in *B* about 2.5 sec. *C*: oscillograph record from optic nerve, stimulus lasting about 1.3 sec.

retina with negative deflections is pathologically changed. The perfect recovery from the negativity obtained above is also difficult to interpret on such a basis.

The discussion of the nature and localization of the components will be postponed till evidence relating to the optic nerve has been presented.

SUMMARY.

Leads from the cornea and decerebration wound have been taken to the input of a directly coupled amplifier with a string galvanometer in the output. The aim of the work has been to try to establish a biological analysis of the complex action potential of the retina. This has been done in two ways: by giving the animal ether and by interfering with the blood supply of the retina. Both agents were found to affect certain components selectively and in a reversible manner.

Narcotization removes in three characteristic steps definite components of the response to stimulation with white light. These components are indicated in Fig. 8 by Roman letters in the order of their disappearance and given separately for a high intensity in Fig. 7. Process I (P I) disappears rapidly during narcotization and the fast deflections are left unchanged. It is essentially a high-intensity component. Thus, at an early stage of anaesthesia, this component may be minute or even absent at high intensities, whereas the low-intensity response is almost or even completely unchanged. Therefore the slow phase of the composite effect is not homogeneous. The positive remainder after removal of P I reacts uniformly and simultaneously to ether at all intensities, diminishing gradually during continued anaesthesia. This component is termed P II. Finally only a negative, P III, is left provided the intensity has been high enough. The last stage is a gradual disappearance of P III. The ether analysis shows the response at low intensities to be a practically pure P II. Removal of P I need not affect it, and when the positive deflection is removed there is no negative left.

Asphyxia in the animal or occlusion of the carotid affects selectively P II. The selectivity may be demonstrated by testing with the practically pure P II at a low intensity. The high-intensity response contains P I and P III, and is a large negative deflection followed by a secondary positive rise.

Removal of P II in this manner shows the brief initial negative (α -wave) running on into the large negative P III of which it is therefore a part.

Removal of P I by ether often enhances the off-effect. Removal of P II by asphyxia regularly enhances the off-effect. The practically pure P II at low intensities never gives an off-effect. Therefore the off-effect depends primarily upon P III. Since, however, P III produces an off-effect only in the presence of either P I or P II it must be resolved by an interference construction from the rise of P III (cf. Fig. 8).

Part II. The latent period and the relation between the processes in retina and nerve.

Action currents from the optic nerve were first successfully recorded by Kühne and Steiner [1881], later by Ishihara [1906] and by Westerlund [1912]. The effect obtained resembles the retinal action potential, even the initial fast *a*-wave being present in the records of Westerlund. In none of the records published can a secondary rise (*c*-wave) be found. Fröhlich [1914] observed upon the retinal action current of the cephalopod eye oscillations which have been interpreted as caused by impulses in the optic nerve, but there are also other explanations to be considered [cf. Kohlrausch, 1931].

The actual impulses in the optic nerve were then recorded in an interesting work by Adrian and Matthews [1927 *a, b*, 1928], who used a capillary electrometer and an amplifier. They used the long optic nerve of the conger eel. Adrian and Matthews confirmed the general relation between intensity of stimulation and frequency of discharge, established by Adrian and his successive collaborators [cf. Adrian, 1928] for various sensory end organs and neurones. They also obtained the frequency-time curve of the retinal discharge. We now know that the frequency of the impulses discharged by the retina first rises rapidly at the onset of stimulation, then falls to a lower level during continued stimulation, and also that the off-effect of the retinal action potential has its counterpart in a renewed outburst of impulses at the cessation of illumination. Considering the slowness of the instruments used by the early workers it is possible that what they recorded was the integrated total frequency-time curve, obtained by Adrian and Matthews by plotting the impulses per unit time against time of stimulation. But it is also quite probable that the effect recorded was due to spread from the retinal currents. The latter view appears to be taken by Westerlund, and my own experiences with "integrative" recording controlled by

oscillograph records taken with large condensers in the amplifying circuit show that "integrative" records may be seriously distorted by retinal effects, at least when the leads are applied as will be described below.

Most important is the observation by Adrian and Matthews that the off-effect also is translated into impulses. This distinguishes the retinal discharge from that of other sensory end organs recorded by Adrian and his co-workers [Adrian, 1928]. Interesting work with the *Limulus* eye has recently been published by Hartline and Graham [1932], who succeeded in obtaining impulses from a single ommatidium. The ommatidium is a fairly complicated structure [Demoll, 1910; Versluys and Demoll, 1922-3], but is not connected with other ommatidia by way of internuncial neurones. However, its internal organization is complicated enough to make it appear questionable whether it can be assumed to be non-synaptic. The retinal action potential of several ommatidia looks like the isolated component P II of the cat's eye and appears to be related to the frequency of the discharge in the nerve [Hartline, 1932]. Further experimentation, no doubt, will show whether it is homogeneous or contains a hidden component of opposite sign and whether this eye gives an off-effect.

In this work the aim is to gather information as to how the components of the retinal action potential, isolated in Part I, are represented in the optic nerve. It has not been possible to accomplish this in a quantitative manner. The cat's optic nerve is rather unaccessible and easily damaged. In order to ensure satisfactory development of all three components of the action potential a great number of fibres must be activated which further complicates the task of recording. But the choice of preparation is fully justified by the fact that the retinal action potential of the decerebrate cat is easily split into components.

METHOD.

For retinal responses the technique has already been described in Part I. The "push pull" battery-coupled amplifier was used in most cases; in later work a new two-stage amplifier, also battery coupled, built on the principles set forth by Chaffee, Bovie and Hampson [1923], was used. With Mazda Pentodes 220, this system gives a base line free from drift and a total amplification of about 50. This is more than needed for work with eyes of decerebrate animals. The same amplifier and string galvanometer were used for obtaining records from the optic nerve with syringe needle electrodes [Adrian and Bronk, 1929], stuck into foramen opticum from the cranial side [Granit, 1932 *a*].

When impulses were recorded the animal in its well-insulated and shielded box was moved into another research room where a Matthews' oscillograph with its amplifying system was set up for other purposes. A Cambridge string galvanometer could be worked alongside the oscillograph, and sometimes this string was also connected to the directly coupled amplifier described above. The stimulating and signalling system could not be shifted as easily as the preparation, and therefore a small lamp, run from an 8-volt accumulator and adjusted by means of lenses to illuminate a large part of the retina, was used in connection with the oscillograph. Records of the retinal action potential showed this illumination to be of the order of magnitude of the high intensities obtained with the other apparatus (cf. Part I). The electrodes were generally silver pins. The two leads were used in various positions relative to one another, but the best results were generally obtained when they were parallel and stuck in obliquely deep into the foramen opticum. The discharge recorded in this manner consists of regular or irregular oscillations dependent upon the degree of synchronization in the fibres concerned. Naturally this index of nervous activity is qualitative rather than quantitative, but some idea about the intensity of the effect can be gained by considering various aspects of the records. A test on artefacts was provided by the fact that the experiments ended with removal, sometimes accompanied by restoration, of the components of the retinal action potential.

The stimulating light was generally switched on by means of a key in its own circuit. This moment was recorded on the plate by a pointer attached to a magnetic short-circuiting device. But in some cases a photographic shutter was employed, and then the on and off of the stimulus were not recorded. In the former case the heating and cooling time of the filament entered into the latency of the on- and off-effects. This, of course, was not the case when the accurate device used with the apparatus described in Part I was used. However, when oscillograph and string galvanometer were worked together an absolute value for the latent periods was not needed, the purpose of this combination being to compare retinal and nerve responses relative to one another. Altogether some fifteen animals were used.

Retinal processes in relation to nerve discharge.

Adrian and Matthews [1927 *a*] also made a first attempt to study the mutual relation between retinal and nerve response. They were, however, seriously hampered by the fact that their condenser-coupled amplifier could only reproduce fast processes correctly; hence, as they

point out, the comparison between retinal and nerve response had to be restricted to the initial and final phases of the two processes. They found that the impulses started during the *a*-wave and that the retinal nerve interval was roughly constant. The latter interval was measured from the beginning of the *a*-wave. They concluded that the initial negative deflection is closely associated with the initial outburst of impulses. That the off-effect in the retinal action potential was found to be correlated with an outburst of impulses has already been mentioned.

In view of the fact that the retinal action potential, as shown in Part I of this paper, is initiated by two processes of opposite sign, their evidence with regard to the *a*-wave hardly allows any conclusions as to which component is associated with the discharge in the nerve. Either the positive component P II, responsible for the *b*-wave, or the negative P III, responsible for the *a*-wave, or both, may be concerned with the initial outburst of impulses. P II may be present at the onset of P III, or P II, if alone assumed to set up impulses, might also start at a constant interval from the beginning of the *a*-wave. It is evident that no records of the complex retinal action potential give any information on this point. The latent period of the *b*-wave merely shows where the rise in P II has cancelled the fall in P III. This moment is probably of some significance in an interpretation of the retinal action potential, but it cannot throw any light upon the relation between the retinal processes and the discharge in the nerve. There was therefore no object in trying to determine the latency of the initial outburst of impulses relative to the beginning of the *a*- and *b*-waves, the less so as in the cat the *a*-wave is small and present only at high intensities. In so far as the onset of the discharge could be measured from records involving a certain amount of synchronization of impulses, it was found to be almost coincident with the onset of the *b*-wave, or to follow not later than 10σ after the first manifestation of a positive deflection, provided that the retinal potential was measured with the string connected to the amplifier.

Not only does indirect evidence support the view that the discharge in the nerve is associated with a positive component, but it also renders it difficult to assign a similar function to the negative wave. One difficulty deserves to be pointed out. The analysis of the retinal action potential (cf. Part I) indicates that the positive off-effect is a release of the positive components from the negative wave, following the return of the latter to zero. Now the retinal off-effect is definitely connected with a discharge of impulses through the nerve [Adrian and Matthews]. If now the *a*-wave, representing the beginning of the negative wave, is

assumed to set up a discharge, it does not seem possible to account for the fact that thereby the negative component is assumed to increase the frequency of the discharge both when P III increases (α -wave) and decreases (off-effect).

The only way in which at present these questions can be approached appears to lie in careful consideration of the various phases of the retinal action potential and how they may be brought to conform with the picture obtained from the nerve. The latter should at least give information on two points: (i) Is there a secondary increase in the discharge corresponding to the large secondary increase in the retinal action potential due to P I (c -wave)? (ii) How does removal of the positive components of the retinal action potential affect the discharge through the nerve?

Fig 9 (Plate III) shows what happens when a purely negative response is produced by interfering with the other waves by the methods described in Part I. Unfortunately, in this case a very small retinal response was obtained with a slack string without an amplifier, but the experiment has the advantage of illustrating both the effect of ether and asphyxia, and also recovery from negativity, in the same preparation. The condensers in the amplifying circuit of the oscillograph were 0.02 microfarad. *A* shows the retinal response at the full intensity; the secondary rise is small. *C* shows the corresponding oscillatory discharge in the nerve. The tendency of these oscillations is to diminish in amplitude during continued stimulation; the frequency may diminish but need not necessarily do so. Values between 100 and 150 oscillations per second are commonly found, the lowest regular frequency noted has been 80. Evidently the discharge consists of synchronized impulses [cf. Adrian and Matthews, 1928; Adrian, 1932]. The frequencies are higher than those noted by Adrian and Matthews in the eel's eye with large areas. If the oscillations noted by Fröhlich are to be similarly interpreted, it is to be observed that the effect of a diminution in intensity primarily affects the amplitude of the oscillations, whereas the frequency decreases but little if at all. Thus *B* is the retinal response at a 100 times lower intensity and *D* the corresponding nerve response, illustrating the diminution in amplitude. Fröhlich found the frequency of the oscillations to increase and decrease with intensity of stimulation. The off-effect in the nerve records is small in this particular experiment, but again the increase in amplitude of the oscillations is noticeable. Thus with large areas in the dark-adapted cat's eye the amplitude of the oscillations is the most definite index of a change in intensity of the retinal effect,

though by no means as good as direct counting of the frequency of the individual impulses where this can be done. It should be noted that there are no indications of a slow secondary rise in the amplitude at the high intensity though the retinal response *A* definitely rises during continued stimulation.

Then the animal is given ether heavily, and in 5 min. the retina has lost its positive components. *E* shows the slow negative deflection in the string. *F* is the nerve record taken immediately afterwards. No oscillations are visible. The anaesthesia is interrupted and 8 min. later the retina gives the response *G*. The oscillations have returned as shown by *H*. Then the carotid is occluded and the negative response *I* is produced in less than 1 min. This is larger than the positive response at the low intensity (*B*). Yet, again no oscillations are visible, though the nerve record, *J*, is taken but a few seconds after the retinal response on the same plate.

Both methods of removing the positive components thus lead to the same result: the impulses disappear. Agents that block the passage of impulses also block the positive components of the retinal action potential. These two processes in retina and nerve respectively are thus closely interrelated. Whether this means that the impulses actually are set up by a positive component or only that the positive components and the oscillations in the nerve are two aspects of the excitatory process as expressed by physiologically and histologically different structures, is at present a secondary question, to be solved, if possible, with different methods.

It is further evident that of the two positive components P II undoubtedly is concerned with the discharge through the nerve. The impulses appear long before there is any sign of the slow secondary rise (*c*-wave) of P I, and, even though the latter is small in this experiment at the high intensity, there is nothing at all to indicate an equivalent rise in the amplitude of the discharge. The off-effect in the cat's retinal action potential is generally only a retardation in the drop of potential following cessation of stimulation, yet this is nearly always accompanied by a corresponding increase in the amplitude of the oscillations. In Part I it was pointed out that the off-effect on the response P II + P III, from which P I had been removed, at times was found larger than in the complex response owing to the fact that in the latter it was compensated for by the fall in P I at cessation of stimulation.

The isolated negative response cannot be shown to set up impulses. This may be accounted for by a block caused by ether or asphyxia, an

explanation difficult to exclude, but not altogether satisfactory. Observations mentioned in Part I showed that an alteration in the amount of potential developed by any component was preceded by a change in latent period. It was further found that a short asphyxia, as in this experiment, did not change the latent period of the negative component. Thus, by this method P III is obtained in as nearly normal form as possible, as indicated by the relatively large negative deflection I (Fig. 9). Considering that the impulses disappear and reappear with P II it is difficult to assume that a negative wave, which hardly is influenced at all by the same amount of asphyxia (1 min. occlusion), should be responsible for the discharge in the optic nerve.

Fortunately the analysis of the retinal action potential demonstrates the nature of the process behind the negative wave, provided that we know the function of P II. This, as we have seen, is to set up impulses or to be a link in the chain of events leading up to the discharge through the nerve. The analysis gives the further information that the off-effect is a release phenomenon, caused by the return to zero of the negative wave. The evidence may be found in Part I. This would be merely an algebraical fact—and is probably so with respect to the response $P I + P III$ (see below)—if we did not know that P II is concerned with the discharge and that the off-effect also is accompanied by impulses. Knowledge of all these facts leads to the conclusion that, when P II is released from the negative P III, it is actually being released from something which not only did not produce impulses, but actively inhibited them. P III can act in this manner only if in some way it is connected with an inhibitory process. The off-effect must then be a true “post-inhibitory rebound,” the term signifying a discharge following as a release from an inhibition. Further evidence bearing on these conclusions will be presented in a subsequent paper by Dr R. S. Creed and the author.

In order to stress the significance of possessing the correct analysis of the retinal action potential and what is chiefly an application of this knowledge to the present problem, the following facts might be pointed out: the *b*-wave and the off-effect are both positive in the retinal action potential, and both are concerned with the discharge through the nerve. In addition the off-effect has also been shown to depend upon the negative wave (cf. Part I). All these facts are taken account of by the above deductions which also are in accordance with the fact that the retinal action potential arises in the synapses (see below).

As to the slow secondary rise given by P I it has already been

mentioned that previous work with the optic nerve has failed to give any indication of a corresponding increase in the frequency of the impulses in the optic nerve. Likewise in this work it has been noted that, whereas a small positive off-effect, visible only as a retardation in the drop of potential at cessation of stimulation, has been accompanied by a definite increase in amplitude of the oscillations, nothing of the kind has been observed with *c*-waves of considerable magnitude. An especially large secondary rise was found in the experiment illustrated in Fig. 10 (Plate IV). *A* and *B* show the retinal action potential at two speeds of the plate. *C* shows the response obtained from the nerve. This was taken with large condensers in the amplifying circuit, combined with a grid leak such as to cause a constant potential applied to the input of the amplifier to drop in the output to half its full value in half a second.

The question arises whether the initial deflection in the nerve and the large off-deflection are artefacts from the retina, or whether the discharge is partly monophasic, in which case the height of the deflection would roughly indicate the frequency of impulses out of phase. Both slow changes ultimately develop into the typical synchronized discharge. Independently of whether the amplitude of the synchronized oscillations alone or these together with the slow rise are interpreted as indicative of the intensity of the effect in the nerve, it is evident that the large secondary rise in the retina has no equivalent effect in the nerve. In this respect it clearly differs from the positive effects in the retina at on and off. Whichever index is used, the off-effect, though only a retardation in the drop of potential at cessation of stimulation, is followed by a marked increase in the intensity of the effect in the nerve. The small secondary rise, to be seen in this nerve record, could not be obtained in the other experiments of the same series. Yet, in one the illumination was allowed to act for over 2 sec. Similarly after removal of P I with ether no definite change can be found, though for such experiments the method is hardly sensitive enough. In some records there has been a small early secondary rise in amplitude which, however, has persisted after removal of P I [cf. Hartline and Graham, 1932]. This rise may be given by P II (cf. Fig. 8, Part I).

The latent period.

A theoretically very important experiment on the latent period was made by Adrian and Matthews [1928]. These authors confirmed an observation, previously made by Ishihara [1906], that an increase in area shortens the latent period just as does an increase in intensity. Actually the latent period of the optic nerve response was measured, but

since the retinal nerve interval was found roughly constant, the process responsible for the shortening of the latent period could be ascribed to the period preceding the retinal response. This latter latency was measured directly by Ishihara. But Adrian and Matthews also added an interesting analysis of this fact by proving that the spatial effect on the latent period could be obtained when areas some distance apart on the retina were stimulated. The latency of the nerve discharge for four lights acting simultaneously was found to be shorter than that for each spot alone. In addition they proved that stimuli so far apart as not to interact could be made to do so by application of strychnine to the retina. This not only excludes any explanation based on scatter of light but also shows that the lateral connections between cells in the retina react to strychnine just as similar connections in the central nervous system. In an eye lacking the internuncial neurones of the vertebrate retina the spatial effect should be absent. Adrian and Matthews' analysis of the spatial effect may therefore be held to have been significantly verified by the observation by Graham—kindly communicated to the author from work in course of publication—that in the eye of *Limulus*, which lacks internuncial neurones, the influence of area on the latent period is absent, though the intensity effect is present.

The spatial effect is of such fundamental importance because it localizes the process responsible for the action potential to a point in the retina which is synaptic or post-synaptic. The first lateral connections are lying around the synapses between receptors and bipolar cells. Thus, since the synaptic effect is to precede the response, the action potential cannot be localized to the rods and cones themselves, but must be produced at the point where the receptors are joined to the bipolar cells or later. There cannot therefore be more than one synapse between the retinal action potential and the impulses in the optic nerve.

This experiment by Adrian and Matthews has been confirmed with the flicker method and the human eye [Granit, 1930], but it was thought desirable to repeat it with the action potential of the cat's eye as well. Four circular discs, 6 mm. in diameter (about 56' of visual angle), were placed symmetrically as outer tangents to an imaginary circle 19 mm. in diameter (nearly 3°) at a distance of 370 mm. from the cat's eye. The intensity was 11.2 millilamberts. The average latency of response to the individual lights in nine determinations was found to be $67 \pm 2.3\sigma$ mean variation. All four together gave $59 \pm 2.0\sigma$ as an average of five determinations. The decrease in latency is thus 11.9 p.c. of the value obtained with the "singles." The latencies were measured with a magnifying glass

placed over a scale reading 0.1 mm., the speed of the film averaging 8-9 scale divisions for 10σ .

In order both to compare this effect with the corresponding variation caused by an increase in area and to find out how much could conceivably be accounted for by scatter of light, the area was varied in the same experiment between limits of 4.5 and 46 mm. in diameter, other conditions being equal. Three readings were taken for each area. The latent periods are plotted in Fig. 11 against log area. The relation is well

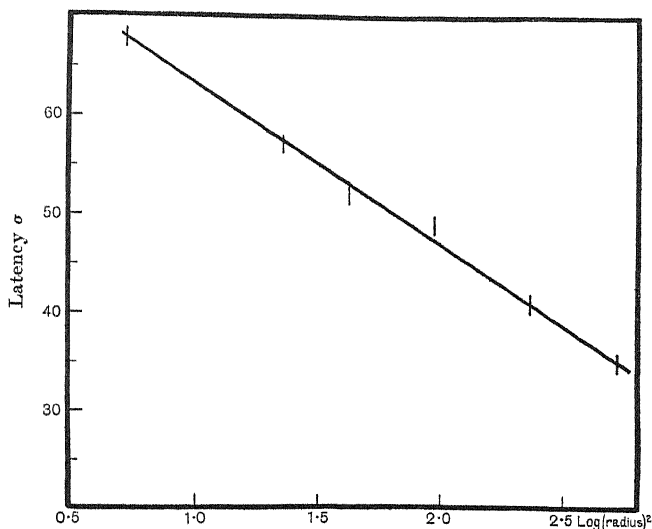


Fig. 11. Ordinates: latent period of *b*-wave of retinal response (no *a*-wave visible). Abscissæ: log area of stimulus. The readings are marked by lines corresponding to ordinates of 2σ . Explanation in text.

represented by a straight line, the one drawn being calculated from the data by the method of least squares. From this graph or from the equation could be read the decrease in latency corresponding to a quadrupling of the area stimulated, given by the four spots together over the area of each single stimulus. The effect evaluated as above is 15.4 p.c. as compared with 11.9 p.c. obtained with separated stimuli. Thus about 77 p.c. would have to be accounted for by scattering of light. This appears an improbable figure, even if the strychnine experiments were not there to disprove this hypothesis.

Intensity influences the latent period in a similar manner [Einthoven and Jolly, 1908; Ishihara, 1906; Adrian and Matthews, 1927 a;

Granit, 1932 *b*]. But within the range of intensities used in these experiments it has always been possible, by diminishing the area, to reach a value of the latency, which could not be compensated for by an increase in intensity. This means that the individual receptor-bipolar synapse at any intensity must have a longer latency than any recorded. The value obtained is largely dependent upon the amount of spatial summation which occurs, that is, upon the area stimulated. The negative initial wave, as pointed out above, also complicates the picture. It is therefore misleading to compare, as Hecht [1931] does, the results obtained by Adrian and Matthews [1927 *b*] on the latent period of the eel's eye with those obtained by himself with the visual end organs of certain invertebrates. Adrian and Matthews did so themselves, but in a later paper [1928], the third in their series, they gave clear proofs that a synaptic factor entered into the latent period of the vertebrate retina. This is not taken into account in Hecht's theoretical considerations, and it may be unnecessary to consider it in the receptor organs which he used.

The latent period in a synaptic structure has been shown to be the time necessary for building up an excitatory state to threshold value [Eccles and Sherrington, 1931], in our case, partly against adverse inhibition. But it is difficult to deduce any information from the latent period of the retinal action potential when it is not even known whether the whole of P II and of P III refers to opposite processes in the same neurones. The presence of an off-effect indicates that part at least of the inhibitory effects is influencing neurones in the act of building up excitation.

Summarizing conclusions.

In the following summary the term "component" (sc. of the retinal action potential) is often substituted for "process," as has been done throughout this paper. But it is important to realize that when two components, measured by the amount of potential produced, are equal, this does not necessarily imply, for instance, that the processes eliciting those potentials are of equal strength. Not only does the picture obtained with the galvanometer depend upon the manner of leading off from the preparation and upon the sensitivity of the instrument employed, but it is also at present impossible to determine whether, to take an example, the frequency of the impulses is proportional to the deflection of the galvanometer with a pure P II or whether it is some more complicated function of the recorded potential. The same holds for P III. A more suitable preparation than the decerebrate cat would appear to be needed for a

study of these questions. It should further be recalled that the traditional way of representing the retinal action potential reverses the sign of the potential with respect to the retinal layers.

The complex retinal potential arising in the retina on stimulation with white light has been found to develop in that part of the sense organ which histologically is a "true nervous centre" [Cajal]. It appears after a latent period involving synaptic interaction, and hence cannot have arisen distal to the locus where the first synapses occur. The complex effect is an algebraical sum of three components, the properties of which may be summarized as follows:

The first process, P I (see Figs. 7 and 8, Part I, pp. 220 and 221), rises slowly after a long latency and falls in a similar manner. It is positive in the usual representation of the retinal action potential. P I is easily removed by ether. Before it begins to diminish it may, however, pass through a temporary stage of enhancement. Likewise after removal of the narcotic it is often temporarily enhanced in good preparations. Slight asphyxia often favours P I. In the dark-adapted cat the first process is only present with large areas and high intensities of stimulation. Observations by Kohlrausch [1918] on the *c*-wave of the complex potential indicate that P I in nocturnal animals is more marked in the dark-adapted eye, in diurnal animals in the light-adapted eye. In order to account for these facts Kohlrausch suggests that this wave appears whenever an eye functions under conditions most appropriate for the particular retina in question [Kohlrausch, 1931]. The dependence of P I upon a large area and a high intensity also suggests that this component appears when the retina is especially active. This component is not at all or only slightly concerned with the discharge of impulses. But it might well represent some process of importance for the maintenance of a continued discharge. Kohlrausch's results appear to exclude pigment or rod and cone movements as possible sources of P I. Its reactions to ether and asphyxia indicate a process of central origin (retinal synapses, cell bodies), perhaps akin to the slow changes recorded by Adrian and Buytendijk [1931] from a central structure (cf. also Birsch-Hirschfeld [1900] for histological changes in the retina after illumination).

The second process, P II, rises rapidly as the positive *b*-wave of the complex response, then falls fairly rapidly at high intensities, less rapidly at low intensities, and continues hidden by the first process under the *c*-wave of the complex action potential. It is the only process that can be detected at all intensities capable of giving a detectable response

and is of the same sign as the potentials recorded from non-vertebrate eyes. This component is associated with the production of impulses. P II is selectively affected by asphyxia and can also be removed with ether during prolonged narcotization. It thus reacts to ether and asphyxia as the negative potential recorded by Gasser and Graham [1932] in the spinal cord which they believe is connected with summation. The fall in the *b*-wave and the similar phenomenon observed by Adrian and Matthews in the frequency of the impulses probably represents, partly at least, a process of adaptation but is also dependent upon

The third process, P III: this is of negative sign and therefore by algebraical summation influences the amount of potential in the complex response. P III first appears as the *a*-wave of the composite potential, its further course is hidden, but by its return to zero at cessation of stimulation the positive off-effect is elicited as a release phenomenon. The off-effect has its counterpart in a renewed discharge through the optic nerve. This is held to imply that when P II is released from the negative P III it is being released from a process in some way concerned with the inhibition of impulses. By definition the off-effect is then a "post-inhibitory rebound." P III is the most persistent of the components of the retinal action potential. A potential wave of opposite sign to the one Gasser and Graham [1932] believed to be concerned with summation was also noted by them in the spinal cord. They suggest that it might be inhibitory in character. The eye performs several functions in which an inhibitory process should be useful [cf. Graham and Granit, 1931], but there is little reason to discuss them as long as this process presents a number of problems, accessible to experimental approach, which should first be solved.

Vision and retinal processes.

The previous pages should have made it evident that our knowledge of retinal physiology still is at the stage when even quite elementary facts have to be established about the nature of the processes concerned. Little can therefore be gained by theorizing extensively about the significance of this work for the subject of vision. It is not even possible at present to express the retinal action potential in terms of frequency of impulses. A hypothesis has been offered by Kohlrausch [1931], but we are clearly far from the stage at which elaboration of theories may prove fruitful.

But it is worth while to make one generalization: this is the necessity of realizing that the retina as a sense organ cannot be identified with

the rod-cone receptor system. The synaptic apparatus continuously modifies the primary response determined by the properties of the receptors. Special methods have to be developed to prove *in casu* that a measured function is due exclusively to, say, the photochemical processes in the receptors. Thanks largely to the work of Sherrington and his collaborators [see *e.g.* Creed, Denny-Brown, Eccles, Liddell and Sherrington, 1932] the physiology of the synaptic reactions has now been developed to a point when real significance may be derived from the fact that such reactions are present in the retina. A case in point is the question of area stimulated. In Part I it was pointed out that the action potential obtained with a small area and a high intensity differs, not only in amount, but also with regard to form and time relations, from the potential obtained when the area is large. Thus the reaction behind the potential has been organically changed to a low-intensity process by a diminution in area stimulated. Probably the number of active units also enters into the total effect in a manner determined by the electrical conditions in the tissue, but more important is the fact that they do so in a purely physiological way, making up a characteristic total reaction by way of processes of interaction at the synapses.

Considering the complications present already at the "sub-sensational stage," studied above, more work with the retina rather than with sensations would appear to be necessary for the establishment of a retinal physiology on a sufficiently broad and unprejudiced basis. Quantitative correlations between sensory phenomena studied through the medium of sensations and certain assumptions as to the nature of the photochemical mechanism of the receptors elaborated, for instance, by Hecht, can hardly give more information about the processes concerned than purely empirical equations.

SUMMARY.

Of the three components of the retinal action potential only one, P II, can be shown to be associated with the discharge of impulses through the optic nerve. P III appears to be related to an inhibitory process. P I does not appear to be concerned with the discharge of impulses, or, if so, to a very small degree. These statements are summarized in greater detail on pp. 223 and 234.

Prof. Sherrington has placed at my disposal the facilities of this laboratory; for this and for his kind active interest in the work I am very grateful.

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EXPERIMENTS ON THE NATURE OF THE
LABILE RÔLE OF SULPHUR IN METABOLISM.

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PREVIOUS experimental work by the writer [Wilson, 1925, 1926, 1931] appears to justify the conclusion that in the catabolism of protein the sulphur fraction is the first to be dealt with: it seems also that in the anabolic phase the sulphur takes a lead, although the evidence on this point is not so striking. The complex nature of the metabolic processes renders the separate analysis of these two phases difficult. They tend in the living cell under normal conditions to keep in equilibrium, and it is only when either anabolism or catabolism is predominant that the liability of the sulphur moiety is apparent. If, however, a metabolite containing sulphur is the mobile unit, the question arises at what phase of its metabolism is its liability exhibited. At present we know too little of the anabolic changes to attempt any investigation of this aspect of the problem. But we can attempt to find out at what stage in protein catabolism the sulphur fraction is most open to attack. The sole evidence, however, in favour of the preferential catabolism of sulphur lies in its early excretion in the urine for which several factors might be responsible. If it be assumed that ingested protein is built up into some complex after absorption and before it is metabolized there are three different phases in the catabolism and excretion of sulphur, any one or any combination of which might be responsible for its early elimination in the urine. In the first place if some complex is built up from the amino-acids derived from the food, in the process of catabolism some of the amino-acids may be released from the molecule before others. This question has not been investigated *in vivo* so far, but Abderhalden [1923] has shown that, in a tryptic digest of protein, tyrosine is split off more quickly than glutamic acid, while cystine is said to appear early in the digest. If this holds good *in vivo*, then the early excretion of sulphur is possibly due simply to the cystine being available for catabolism before the majority of the other amino-acids. In the second place, however, it is possible that free cystine is much more readily catabolized than the other amino-acids, hence the excretion of sulphur in the urine would tend to precede

the nitrogen. There is yet a third possibility, namely, that all the amino-acids are equally liable but that the sulphates are more quickly eliminated by the kidney than urea or ammonia. It is to be noted that the two latter possibilities might explain the early excretion of sulphur in catabolism but they would not explain the behaviour of sulphur in anabolism.

The first possibility, on the other hand, might be invoked to explain not only the preferential catabolism of sulphur but also its leading rôle in the anabolic phase, if it could be proved that in protein synthesis the sulphur is the first to be built up into the protein molecule.

Experiments were therefore planned to find out if either of the two latter hypotheses were true in regard to the catabolism of the sulphur fraction. The question of the preferential excretion by the kidney of sulphate over urea was first investigated. The experiments on this aspect of the problem were carried out on a female dog weighing 14 kg. The principle of the method was to feed ammonium sulphate and note the relative rate of excretion of sulphur and nitrogen. It is known that ammonium sulphate is changed in the body and excreted as an alkaline sulphate, while most of the ammonia is combined with CO_2 to form urea, although some of it may be excreted as such. The animal was kept in a metabolic cage during the course of the experiments and the urine was drawn off by catheter. In Exp. 1 (Table I) the animal was fasted for 3 days, and on the morning of the fourth day a solution of ammonium sulphate was given by mouth. Owing to the fact that a stomach tube could not be passed in this particular animal an exact record of the amount of the salt given was not obtained. This, however, does not invalidate the results as the object was to determine the Sulphur:Nitrogen ratio of the excess material

TABLE I.

Period	Diet	Total N g.	Total S g.	S : N	Excess N g.	Excess S g.	S : N of excess
EXP. 1.							
I (6 hr.)	Fasting	0.753	0.034	1 : 22.14	—	—	—
II (6 hr.)	$(\text{NH}_4)_2\text{SO}_4$ administered	1.484	0.413	1 : 3.59	0.731	0.379	1 : 1.92
III (6 hr.)	Fasting	1.550	0.351	1 : 4.41	0.797	0.317	1 : 2.51
IV (12 hr.)	"	1.121	0.208	1 : 5.38	—	0.070	—
IV (average per 6 hr.)	"	0.560	0.104	1 : 5.38	—	—	—
V (6 hr.)	"	0.460	0.035	1 : 13.15	—	—	—
EXP. 2.							
I (24 hr.)	Basal 1	2.748	0.126	1 : 21.80	—	—	—
II (24 hr.)	Basal + 7 g. $(\text{NH}_4)_2\text{SO}_4$	4.048	1.264	1 : 3.20	1.300	1.138	1 : 1.14
III (24 hr.)	Basal	2.987	0.216	1 : 13.82	0.239	0.090	1 : 2.65

excreted over that in Period I. In order not to miss the early excretion of sulphur, the urine was collected in two periods of 6 hours following the administration of the salt.

In Exp. 2 (Table I) so as to induce the animal to take a larger quantity of the salt a standard diet was fed daily, and on the experimental day 7 g. of the salt were added. The urine in this experiment was collected over the 24 hours. The excess nitrogen and sulphur (hereafter N and S) and the S:N ratios were calculated, using the figures in Period I as basal values. The S:N ratios of the excess were then compared with that of the ingested salt. The standard diet employed had the following composition: 60 g. fat, 100 g. beef, 100 g. tapioca, 10 g. bone ash.

It will be noted that in Exp. 1 (Table I) the ratio of the excess is 1 : 1.93 in Period II and 1 : 2.5 in Period III. In the subsequent 12 hours the N output was below the basal value, while S was still being excreted. There is no indication of the preferential excretion of S as judged by the S:N ratio of the excess which does not even reach the theoretical for the salt ingested (1 : 0.87). The figures would have been more striking if Period V had been chosen, for the basal values as the N output is lower than in Period I, while the S is almost identical. In Exp. 2 (Table I), in a 24 hours' period, the same phenomenon was noted, and it may be concluded that the factor responsible for the early excretion of S in protein metabolism is not a selective excretion of sulphur.

A series of experiments were therefore planned to test the second possibility, namely, that cystine is more readily metabolized than the other amino-acids. If the food protein is absorbed and catabolized directly as a mixture of amino-acids, a comparison of the rate of elimination of S after cystine ingestion with the rate of elimination of N after a protein has been superimposed, should show whether cystine is catabolized more quickly than a mixture of S-free amino-acids. The principle of the method was similar to those previously recorded [Wilson, 1931]. The experiments were carried out on the writer as subject. A basal diet of bread and cheese, butter and jam was ingested until equilibrium was attained and the protein was superimposed for one day. The proteins employed were beef 250 g., gelatin 70 g. and beef 250 g. (Exps. 3, 4 and 5, Table II). The excess N and S outputs were calculated in the usual way, the basal values being obtained from the average of the first two and the last two days of the experimental period (14th, 15th, 30th and 31st Jan.). The percentage of the total excess excreted daily has been calculated for each experiment separately (the total excess excreted over the basal period for each experiment has been taken as 100 and its distribution over 2 to

TABLE II. (Exps. 3, 4 and 5.)

Day	Diet	Total N g.	Total S g.	Excess N		Excess S	
				N above basal g.	excreted daily p.c.	S above basal g.	excreted daily p.c.
1	Basal	7.882	0.491	—	—	—	—
2	„ Exp. 3 Basal + 250 g.	7.627	0.480	—	—	—	—
3	Beef	10.301	0.739	2.303	70.5	0.239	87.8
4	Basal	8.960	0.533	0.962	29.4	0.033	12.2
5	„	7.784	0.464	—	—	—	—
6	„	7.952	0.454	—	—	—	—
7	„ Exp. 4 Basal + 70 g.	8.072	0.524	—	—	—	—
8	Gelatin	11.961	0.659	3.963	55.9	0.159	65.9
9	Basal	9.996	0.533	1.998	28.2	0.033	13.6
10	„	8.562	0.516	0.564	7.9	0.016	6.6
11	„	8.554	0.533	0.556	7.8	0.033	13.6
12	„	7.851	0.511	—	—	—	—
13	„ Exp. 5 Basal + 250 g.	8.285	0.533	—	—	—	—
14	Beef	11.544	0.791	3.546	46.4	0.291	63.6
15	Basal	10.301	0.607	2.303	30.1	0.107	23.4
16	„	9.293	0.527	1.295	16.9	0.027	5.9
17	„	8.201	0.527	0.203	2.6	0.027	5.9
18	„	8.282	0.505	0.284	3.7	0.005	1.0

5 days calculated). It should be noted that the assumption in these experiments is that ingested protein is absorbed and metabolized as a mixture of amino-acids. The main object of the experiments was simply to observe the rate of excretion of N alone. The S excretion, however, has been given in addition as it may also be compared to the S output after cystine ingestion. It will be seen in these three experiments that the percentage of S eliminated on the day of ingestion is higher than that of N. This again confirms what has been repeatedly observed, the preferential catabolism of S. It will be noted also that the excess N and S metabolized in Exp. 3 is relatively small, and consequently the percentage excreted on the day of superimposition is high. In Exp. 5 also with beef the excess metabolized is greater and is spread over 5 days. In Exp. 4 with gelatin the excess excreted is spread over 4 days. It will be seen that the percentage of both N and S excreted each day varied for the different experiments according to the number of days over which the catabolism of the ingested material was spread.

It remains now to compare those figures with the rate of excretion of S after cystine ingestion. A nitrogen-free diet (Exp. 6, Table III), similar to that previously employed by the writer, was taken, and on the sixth day 4 g. of cystine containing 1.060 g. S and 0.564 g. N were superimposed. The excess S was calculated by employing the S output on the

TABLE III.

EXP. 6.

Day	Diet	Total N g.	Total S g.	S : N	Excess S g.	Excess S excreted daily p.c.
1	Basal N-free	8.232	0.439	1 : 18.72	—	—
2	" "	5.241	0.291	1 : 18.01	—	—
3	" "	4.558	0.274	1 : 16.63	—	—
4	" "	4.172	0.258	1 : 16.17	—	—
5	" "	3.864	0.247	1 : 15.63	—	—
6	Basal + 4 g. cystine	4.816	0.835	1 : 5.76	0.588	47.53
7	Basal	4.480	0.555	1 : 8.07	0.308	24.89
8	" "	4.064	0.500	1 : 8.12	0.253	20.45
9	" "	4.648	0.335	1 : 13.87	0.088	7.11

EXP. 7.

Day	Diet	Total N g.	Total S g.	S : N	Excess N g.	Excess N excreted daily p.c.	Excess S g.	Excess S excreted daily p.c.
1	Basal N-free	4.065	0.348	1 : 11.68	—	—	—	—
2	" "	3.654	0.268	1 : 13.63	—	—	—	—
3	Basal + 4 g. cystine + 250 g. beef	6.258	1.044	1 : 5.99	2.604	51.52	0.776	62.37
4	Basal	5.348	0.621	1 : 8.61	1.694	33.51	0.353	28.37
5	" "	4.410	0.368	1 : 11.98	0.756	14.95	0.100	8.03
6	" "	3.654	0.283	1 : 12.91	—	—	0.015	1.20

day before as a basal value. Over the 4 days 1.237 g. of excess S were excreted, and hence there was a small negative balance. The percentage of the total excreted on the day of superimposition was 47.53 p.c. and it fell to 7.11 p.c. by the fourth day. If these figures are compared with the percentage N excretion on each day after protein ingestion (Exps. 3, 4 and 5), it will be seen that they are less than those in Exps. 3 and 4 and approximately the same as those in Exp. 5. In Exp. 7 (Table III) 4 g. cystine and 250 g. beef were superimposed for 1 day on a similar N-free diet. It will be noted that of the total excess S excreted, namely 1.244 g. 62 p.c. has been eliminated on the first day and 1.2 p.c. on the fourth day. It should be observed that the total excess S is scarcely greater than in the previous experiment in spite of the fact that an extra 0.55 g. S was ingested as beef along with the cystine. Apparently the rate of elimination of the excess S has been accelerated by the metabolizing of the beef, as the percentage of the total excess S on the day of superimposition in Exp. 7 is 62.37 p.c., as opposed to only 47.53 p.c. in Exp. 5 with cystine alone, and this in spite of the fact that the total excess S excreted in each experiment is approximately the same, although an extra 0.55 g. S was ingested as beef in Exp. 7. It is probable, however, that this increased percentage of S excreted on the day of superimposition in Exp. 7 is due to the S of the beef being metabolized in advance. In Exp. 5 (Table II) with beef alone the percentage excretion on the day of superimposition

was 63·6 p.c. as compared with 62·37 p.c. in Exp. 7 where cystine and beef were ingested.

The evidence from these experiments does not support the hypothesis that cystine is more readily catabolized than any other amino-acids. This might be expected in the light of Lewis and Root's work [1922]. These investigators have shown that cystine is not metabolized unless its amino-group has been removed. In other words deamination precedes oxidation of the sulphur group. If the process of deamination is the same for all the amino-acids it would be expected that all those acids including cystine should be of equal lability. In view of the evidence which shows that the sulphur moiety is possibly the mobile unit both in anabolism and catabolism and further that, as far as catabolism is concerned, the labile phase is not in the final process of demolition of the amino-acid, it would appear that a final solution of both aspects of its metabolism will be found in its position in the protein molecule or even the living protoplasmic unit. In the breakdown of protein the sulphur might be split off first, and hence it would be available for catabolism and early excretion in the urine. The preferential retention of this element might correspondingly be due to the unit containing sulphur being taken up to form perhaps not the nucleus but possibly the keystone of the protein molecule. The sulphur moiety would hence both confer and condition the stability of the biological unit. Its lability might, therefore, be due not to any inherent property of, say, cystine but rather to the position it takes in relation to the other constituents of the protein molecule. In this connection some observations of Hopkins [1930] are of interest. He showed that one of the features of denaturation of egg albumin is the appearance of the thiol group as shown by the nitroprusside reaction. This opens up the possibility that, in the catabolism of protein, some molecular change may take place whereby the sulphur fraction is more readily oxidized or split off from the protein molecule. A sulphur grouping, at all events, appears to be in a readily accessible position in the protein molecule.

SUMMARY.

1. A series of experiments were carried out in order to elucidate at what phase in the metabolism of protein the liability of the sulphur moiety is apparent.

2. Three possible phases are discussed: (1) that the early excretion of sulphur in the urine is due to the kidney excreting sulphates more

readily than urea or ammonia; (2) that cystine is more open to attack than the other amino-acids; (3) that cystine or some unit containing sulphur occupies a key position in the protein molecule whereby it is the first to be split off in catabolism and the first to be retained in anabolism.

3. The experimental results do not favour the view that the first two possibilities can explain the phenomena. The last hypothesis is held to explain most satisfactorily the labile rôle of sulphur in both phases of metabolism.

I desire to acknowledge my indebtedness to Prof. Cathcart for much helpful advice and criticism during the progress of the work.

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THE ANTAGONISTIC EFFECT OF ALCOHOL ON PITUITRIN HYPERGLYCÆMIA.

By M. M. MURRAY.

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IN a series of experiments on normal human subjects Edkins and Murray [1931] showed that the ingestion of alcohol with glucose decreased the degree and duration of the hyperglycæmia. Hunt [1930], in diabetic patients, showed that alcohol definitely decreased hyperglycæmia. In the experiments on normal subjects the degree of hyperglycæmia when alcohol and sugar were taken together seemed to be related to the amount of diuresis. When the hyperglycæmia was small the diuresis was big and *vice versa*. As a result of this relationship a suggestion was put forward that a possible explanation might be, that alcohol inhibits the secretion of the posterior lobe of the pituitary gland, or in some way prevents the development in the body of its ordinary action. Injections of an extract of this lobe ("pituitrin") are known to produce hyperglycæmia, and also antidiuresis or diuresis according to the conditions of experiment. In a more recent paper Murray [1932] has shown that the ingestion of alcohol antagonized the effect of pituitrin injection on water diuresis in normal human subjects; and in anæsthetized cats diminished the diuretic response to pituitrin. The experiments to be described here were designed to test the effect of alcohol on the hyperglycæmic response to pituitrin injections.

Cats, kept on a generous diet of meat and milk for at least a week, were deprived of food for 18 hours before the experiments. They were given intra-peritoneal injections of amytal with no preliminary anaesthesia. The carotid artery on one side was exposed for the taking of blood samples, the puncture in the artery being clipped without occlusion of the artery. A tube was tied into the oesophagus for the administration of liquids. The hyperglycæmic effect of pituitrin in cats has been systematically investigated by Clark [1927], and shown to be due to release of glycogen from the liver. It was not possible to compare the effect of pituitrin in the same animal before and after alcohol, because the liver glycogen continually decreases in animals under amytal, and also because one response

to pituitrin might diminish the effect of a second injection. This difficulty was surmounted to a certain extent by making a large number of experiments; these can be divided into four groups:

- (1) Hyperglycæmia due to a single injection of pituitrin.
- (2) Hyperglycæmia due to a single injection of pituitrin after giving alcohol.
- (3) Hyperglycæmia due to two successive injections of pituitrin.
- (4) Hyperglycæmia due to one injection of pituitrin and then a second injection, alcohol being given between the injections.

Blood samples were taken and the sugar estimated until the blood sugar was constant. The estimations were made by the method of Hagedorn and Jensen. The dose of pituitrin, 0.5-0.75 c.c. of Parke-Davis pituitrin (1 c.c. = 20 units), was injected into the saphena vein. When alcohol was given, the amount was 20 c.c. of a 20 p.c. solution, this was introduced into the stomach through the tube in the œsophagus. The results of the injections of pituitrin on the blood sugar under the four different conditions set out above are shown in Table I.

TABLE I. The rise in blood sugar in mg./100 c.c. caused by an injection of 0.5-0.75 c.c. of pituitrin.

No. of Exp.	Pituitrin, first dose	Pituitrin, first dose after alcohol	Pituitrin, second dose	Pituitrin, second dose after alcohol
1	66	—	—	—
7	180	—	—	—
2	—	-30	—	—
3	—	31	—	—
4	—	4	—	—
5	—	19	—	—
29	—	33	—	-20
6	76	—	—	19
10	99	—	—	5
12	49	—	—	4
13	44	—	—	11
16	60	—	—	0
27	55	—	—	11
22	—	—	—	-12
8	102	—	70	—
15	86	—	145	—
36	78	—	85	—
35	123	—	65	—
33	86	—	20	—
32	87	—	40	—
Average	85	11	71	2

From these results it can be seen that after giving alcohol an injection of pituitrin, whether a first or second dose, does not evoke a characteristic hyperglycæmic effect. The way in which alcohol suppresses the rise of

blood sugar is not clear. One possibility is that the alcohol, during absorption, extracted something from the intestinal wall which was carried into the blood and prevented the action of pituitrin. To test this point in a number of experiments the alcohol was given intravenously. In the first few experiments of this kind small doses of alcohol were given, and in these a considerable rise in blood sugar was obtained, so that it seemed that alcohol acted differently according to the mode of administration. In later experiments where the alcohol in the blood was estimated it was obvious that these doses had been too small. When the alcohol was given by stomach tube the alcohol in the blood often attained the value 125–150 mg./100 c.c., a figure obtainable in man by the drinking of about a litre of 8 p.c. alcohol. To get this concentration by injection, almost as much was required as by mouth. This is probably because the high concentration of alcohol in the blood in the first few minutes after the injection leads to a very rapid penetration into the tissues, and also because, when given by mouth, the absorption is rapid and complete and the rate of oxidation very slow. The suitable injection dose was about 15 c.c. of 20 p.c. alcohol in saline. In the experiments where this large dose was given no hyperglycaemia was obtained after injecting pituitrin. Hence the determining factor is definitely the concentration of alcohol in the blood. The results in Table II, relating concentration of alcohol and rise of sugar

TABLE II. Relationship between concentration of alcohol in blood and the rise in blood sugar level caused by pituitrin injection.

No. of Exp.	Conc. of alcohol in blood mg./100 c.c.	Rise in blood sugar mg./100 c.c. after pituitrin, first dose	Rise in blood sugar mg./100 c.c. after pituitrin, second dose	Remarks
40	196	10	— 11	Cat 3.3 kg. 16 c.c. 20 p.c. alcohol intravenously
42	145	16	18	Cat 3.3 kg. 15 c.c. 20 p.c. alcohol intravenously
41	79	128	35	Cat 3.7 kg. 14 c.c. 20 p.c. alcohol intravenously
39a	46	86	—	Cat 2.75 kg. 7 c.c. 10 p.c. alcohol intravenously
39b	93	—	40	Further dose of 10 c.c. 20 p.c. alcohol intravenously
38	154	24	—	Cat 3.0 kg. 20 c.c. of 20 p.c. alcohol by mouth

level in the blood in response to pituitrin injection show this to be so. It seems that alcohol, then, diminishes and checks hyperglycaemia. Small doses given to normal human beings lower the blood sugar slightly

[Edkins and Murray, 1931]. In the Allen treatment of diabetes, whisky was often given because it diminished the glycosuria. The points arise then as to how alcohol reduces and prevents hyperglycæmia, and what is the fate of the sugar. Experiments are being carried out to test whether alcohol prevents adrenaline hyperglycæmia, and also to determine the effect of administration of alcohol on the glycogen content of the liver.

SUMMARY.

Alcohol administered to cats by mouth or intravenously, so that a concentration of over 100 mg./100 c.c. of blood is attained, prevents the rise of blood sugar which normally results from the injection of pituitrin.

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OBSERVATIONS ON SENSATION.

The sensory functions of the skin for touch and pain.

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RESEARCH upon cutaneous sensation in Man during recent years has been directed largely to the study of the results which follow immediately upon experimental division of one or more cutaneous nerves in the observer's own person, and of the gradual recovery of the different forms of sensation—touch, pain, pressure, temperature. These phenomena are puzzling, and different observers are not agreed upon their significance and the interpretation to be put upon them, or on the light which they throw upon the nature of the normal sensory apparatus.

H. Head [1905], from the study of the results of section of two cutaneous nerves in his own arm, put forward his widely quoted theory of the presence of a dual sensory mechanism, each subserving certain forms of sensation and having distinctive characters, generally expressed as a "protopathic" or crude "defensive" sensibility, which serves for the perception of pain and of extreme degrees of temperature, tending to radiate, and an "epicritic" sensibility, presumed to be phylogenetically a later acquisition, which includes what is termed tactile sensation and the discrimination between moderate degrees of temperature.

Head's observations and the conclusions which he drew from them have recently been criticized drastically by Sharpey-Schafer [1929], from the results following nerve section and nerve crushing on his own person. He reached the conclusion that Head's "protopathic" nerve fibres are nothing more than the ordinary "pain fibres" reacting in a peculiar manner from the altered conditions produced by the operation on the nerve trunk and by the growth of the axis cylinders.

The difficulties experienced in interpreting the results of such experiments are without doubt due to our ignorance of the normal mechanism of the production of the different forms of sensation. This is especially

the case in regard to pain, but it holds also for tactile sensation, for temperature sensation and for pressure. Touch is spoken of as having its receptors in the corium, pain as arising from nerves of the epidermis. (In view of the facts described here these statements are erroneous.)

The following observations are a continuation of those on touch and temperature which have been already published [Waterston, 1923]. The training undergone preliminary to these former observations and the experience gained in carrying them out have been of great value. Those who have attempted observations on sensation are agreed that a long preliminary training is essential if accurate and reliable results are to be obtained.

The sensations dealt with here are those of touch ("tactile sensation") and of cutaneous pain, the first the sensation excited from the surface of the skin by light touch with a fine bristle or brush drawn lightly over the surface or applied to individual points.

Pressure is not a cutaneous sensation, it is not elicited by pressure applied to the surface of a fold of skin and subcutaneous tissue, but it is excited by pressure on the surface if sufficiently firm to affect deeper tissues such as muscle or periosteum, etc., and its consideration may be excluded here. Cutaneous pain was tested by the prick of a sharp needle.

It has been shown by many writers that the nerves of the epidermis arise from medullated fibres which form the subpapillary plexus and they lose their medullary sheath when they enter the epidermis, run near one another almost vertically and can be traced to the uppermost layers of the epidermis. Two main varieties of fibres have been found. Some are thick with few varicosities in the deeper layers, and in their course through the stratum mucosum they give off a number of lateral branches which end in small knobs. Other extremely thin fibres are present also, of such fineness that they can be detected only with the highest powers of magnification and can hardly be distinguished from nerve fibrils. These also give off lateral branches [Stöhr, 1928].

Some time ago I put forward evidence [Waterston, 1931] to support the view that the receptor for tactile sensation is in the deeper layers of the epidermis, that some of the cells of that layer are the receptors for touch and that the nerves of the epidermis are tactile nerves. The evidence upon which this view was based lay in the facts that the anatomical arrangement of the parts was appropriate to such a function, that the sensation of touch cannot be elicited from a skin surface denuded of epidermis and that the stimulus adequate to elicit the sensation of touch

in the normal skin is so slight as to render it almost impossible that the deeper organs of the corium (Pacinian bodies, Meisner corpuscles, etc.) can be the receptors for touch as is generally stated. The investigation has now been carried further and the conclusion reached that not only are the nerves of the epidermis the nerves of touch, but also that they are nerves of that specific sensation alone and that they do not convey impulses which give rise to pain. The pain mechanism is separate and distinct and lies in a deeper layer of the skin. It was pointed out in a former paper [Waterston, 1931] that, in the living person, epidermis could be cut away through its deeper layers (*stratum mucosum*) without eliciting the slightest pain but only a sensation of touch, even though, as might be inferred, nerve fibres were divided in making the cut. To complete the evidence it was felt that it was necessary to show that this actually occurred, and search was made to discover if this were so. The method employed was to shave off a portion of cuticle as deeply as to the level of the *stratum mucosum* and deeply to the *stratum granulosum*. The colour of the intact skin is modified by the granules of the *stratum granulosum*, and when that layer is removed the exposed surface is of a bright pink colour. It was found necessary to remove the portion of skin to be examined by a single cut to the required depth rather than by shaving it away in successive slices, for while thin sections can be cut as deeply as to the *stratum granulosum*, they cannot be obtained from the normal skin at a deeper level for the cuticle comes away in small fragments. Section of the skin as described were made in several individuals and in different regions with uniform results. It can be done, however, only in regions where the epidermis is of sufficient thickness.

In the forearm, for example, the epidermis is so thin— $1/20$ or $1/30$ mm. in thickness—that it cannot be shaved away without cutting into the corium and producing pain.

The front and sides of the terminal part of the fingers provide the most suitable site, for there the epithelium is sufficiently thick to allow slices to be shaved off without cutting into the corium.

The areas where sections were made were tested for their sensitiveness to different forms of stimulation, and were responsive to touch, to heat and to cold as well as to pain while the skin was intact.

During the removal of the epidermis the only sensation elicited was that of touch; no pain at all was felt.

Examination of the surface exposed by removal of the greater part of the epidermis showed that the sensation of touch could be elicited from it by the same form of stimulus as before. The sensation, however,

was not entirely the same, it was less definite, more of a prickly nature. Description of the difference is not easy, but the experience of the different persons examined was the same, and the sensation was described as not the same as from the untouched skin.

Possibly all the tactile nerve terminals had not been removed, and some remained behind in the deeper layer of the epidermis, but the explanation of the difference in sensation is probably to be found in the fact that on the denuded area the contact stimulus was applied directly to the divided ends of the tactile nerves and not to the "receptors" with which they are normally in contact.

The small size of the denuded area rendered it unsuitable for the precise investigation of temperature perception on its surface, and it was impossible to identify on the denuded surface the exact position of any touch or temperature spot which had been detected on the epidermal surface while it was intact. So far as could be made out, both cold and warmth could be felt on it, but warmth very quickly passed into pain which obscured the former sensation.

The portions of epidermis removed were stained with gold chloride and lemon juice (Ranvier's method), embedded in paraffin and cut usually vertical to the surface, but in some specimens parallel to it. Microscopical examination of the sections showed that the cut had gone to the required depth, the portions removed including the stratum granulosum and a considerable thickness of the stratum mucosum, which it had traversed superficial to the tips of the papillæ of the corium.

Excellent histological pictures were afforded of the layers of the stratum granulosum, whose granules show up well with this method (gold chloride and acid) of staining.

Since many investigators have demonstrated the presence of fine nerve fibres in epidermis, it is not surprising that we have found nerve fibres and their terminals present in the sections of the portions of skin which we have removed. The fibres are fine, their course is tortuous and they were found only in short lengths in any one preparation. They appeared as short thin rods, lying between the epithelial cells, deeply stained of a dark purplish colour, in some cases slightly varicose, in others with small branches (Fig. 1). They were found in the stratum mucosum and extended as far as to the deep surface of the stratum granulosum. The appearances which they presented were similar to those figured by Ranvier [1880] and to Kadanoff's figures [1924] of nerve fibres among the epidermal cells of the pig's snout, though they were much fewer in number in our preparations.

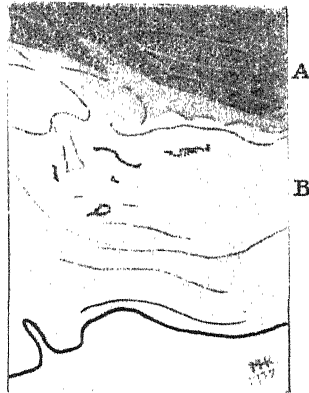


Fig. 1.

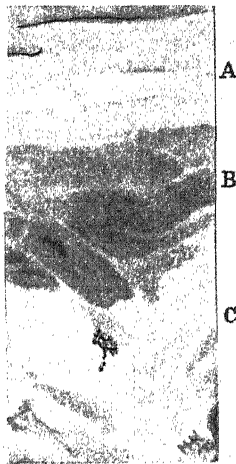


Fig. 2.

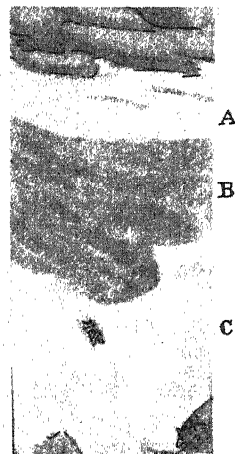


Fig. 3.

Fig. 1. Afferent nerve fibres and a terminal in the stratum mucosum of a piece of removed epidermis. Gold chloride and lemon juice. Camera lucida. $\times 340$. (A) Stratum granulosum. (B) Stratum mucosum.

Fig. 2. Afferent nerve terminal in the stratum mucosum of a portion of removed epidermis, showing its position close to the stratum granulosum. The varicose nerve fibre ends in an arborescent figure on the surface of a cell of the stratum mucosum. Gold chloride and lemon juice. $\times 340$. Camera lucida drawing, with the detail completed with $1/12$ in oil-immersion lens and Swift "C" ocular. $\times 600$. (Section slide W 3, section 3.) (A) Stratum lucidum. (B) Stratum granulosum. (C) Stratum mucosum.

Fig. 3. Next adjacent section of the same terminal, showing the fibrils of the arborescent figure. (Section slide W 3, section 2.)

More distinct than the nerve fibres themselves were the loops and figures in which they terminate. Terminals of this nature have been figured by Kadanoff, and our preparations showed similar appearances. They form arborescent figures of fine threads, slightly varicose, on the surface of epithelial cells in the stratum mucosum (Figs. 2, 3), and they constitute one type of termination of afferent nerves in relation to their receptors, which in this case are some of the cells of the stratum mucosum.

The nerve fibres and the terminals are exceedingly fine and are to be found only with high powers of magnification, and the use of an oil-immersion lens is usually necessary for their satisfactory demonstration.

This demonstration of the presence of nerve fibres and of their terminals in the tissue which had been removed, without causing any pain, completes the evidence necessary to establish the theory which I have put forward, viz. that the nerves of the epithelium are the nerves of light tactile sensation, and that they do not under ordinary conditions convey impulses which give rise to pain. If so severe a form of stimulation as division of these nerve fibres does not give rise to pain, it seems impossible that any other form of stimulus can do so. We should, in other words, regard the epidermis as the receptor tissue for the sensation of touch, with its "receptors" in the deeper layer of epidermic cells and its specific nerve fibres which, like other nerves of special sense, do not give rise to pain even when they are divided. The sense of touch follows Johannes Müller's law, and impulses transmitted by its fibres give rise to no other sensation than the specific special sensation. As the optic nerve can be divided without causing pain, similarly cutaneous tactile nerves can be also without giving rise to pain.

If the evidence now obtained of this theory be accepted it will be necessary to abandon two views which are widely quoted, viz. that nerve fibres of epithelium subserve the sensation of pain, and that the Pacinian bodies or Meisner corpuscles are the receptors for light touch. Both of these theories are now untenable, and other functions must be sought for these and other similar organs of the corium.

Sensibility to superficial pain on the other hand is subserved by the corium. With the twofold structure of the skin, epidermis and dermis, there is a division of sensory function, the epidermis the organ of touch, the corium that of superficial pain.

The conclusion reached here, that the nerves of touch in Man do not convey pain-producing impulses is in harmony with the current physiological view that alterations in the frequency of impulses in afferent nerve fibres modify the intensity, but have no influence whatever on the

quality of the sensation, and confirm and amplify the conclusion reached by Adrian [1932], from investigation of the nerve impulses in cutaneous nerves in the frog and guinea-pig, in which he states that in those animals "the receptors for touch are supplied by the larger medullated fibres and that in some at least of these fibres no messages can give rise to pain in the normal animal."

Miss M. H. Kidston, was responsible for the preparation of the sections and making the drawings from them, and I am greatly indebted to her for the careful work which she has done in this connection.

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SINGLE-SHOCK EXCITATION AND INHIBITION OF
CONTRALATERAL EXTENSION IN THE SPINAL CAT.BY K. MATTHES¹ AND T. C. RUCH².*(From the Department of Physiology, Oxford.)*

AN induction shock given alone and unrepeatd is a relatively ineffective stimulus for the crossed extensor reflex. Yet even in the spinal preparation (cat) we have found, provided the spinal transection has been performed aseptically some days previously, such a stimulus does in the majority of "chronic" spinal preparations suffice to evoke a contraction in the contralateral extensor muscle.

METHOD.

Spinal transections were performed at the level of the first lumbar or last thoracic segment under deep anæsthesia and with full precautions against sepsis. Post-operative care consisted in regular emptying of the bladder and expression of the fæces by manual pressure, and, particularly when the animal was incontinent, of cleaning and drying the hind-quarters. If care is taken, spinal cats can be kept in a healthy condition for almost any desired period. In the experiments recorded in this paper twenty-six chronic spinal cats were used. The operative wound healed by first intention except for two instances. The time elapsing between spinal transection and myographic examination varied between a few days and 3 months.

Preliminary to myographic examination, the animals were decerebrated by the trephine method under full ether anæsthesia, all muscles of the hind-limbs except the one under examination, *M. soleus*, were paralysed by nerve or tendon section, and the preparation rigidly fixed for isometric recording by means of drills through both heads of the bone caught securely by clamps affixed to the iron top of a heavy table. The myograph used was the "frictionless" pattern of the isometric mirror myograph arranged for simultaneous shadow recording of a galvano-

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meter string, signals, etc., as described by Sherrington [1930]. In some experiments the myograph was the steel torsion-strip shadow recording type. Muscle action currents were led by Ag:AgCl pin-electrodes to a Cambridge string galvanometer. By means of a Lucas pendulum, electromagnetically released from the falling-plate camera, single break-shocks from two independent coreless induction coils were applied to the nerves through glass-shielded electrodes. For studying inhibition, the knock-down keys were wired so that stimuli to an ipsilateral and a contralateral nerve could be delivered at all intervals apart up to 465σ . The order of their fall was reversible.

Latent periods of single-shock reflexes were recorded by a derivation of the primary current of the exciting inductorium passing through the same galvanometer string that received the muscle action currents. The derivation shunting the galvanometer string was given a resistance of 10,000 ohms to avoid impairing the sensitivity of the string. The latent period was then directly measured from the string signal to the beginning of the action current.

RESULTS.

The characteristics of the single-shock crossed extensor reflex in spinal chronic animals to which particular attention has been paid include (a) latency, (b) magnitude, (c) duration, and (d) behaviour to single-shock ipsilateral inhibition.

(a) Latent period.

The latency of the single-shock crossed extensor reflex of *soleus* throughout a series of chronic spinal cats varied considerably from animal to animal. The latent period measured by a derivation of the primary circuit of the induction coil through the galvanometer string used as a signal typically lies within the range of $20-35\sigma$ for the shortest latency recorded from each preparation. It may, however, reach the very low figure of 8.7σ ; and the shortest latent period recorded from some preparations was as long as $50-60\sigma$ even when the reflex was of good magnitude.

The shortest latent period encountered (8.7σ) was from a preparation in which the spinal cord had been transected 84 days previously, and had throughout exhibited a marked extensor activity. The brevity of latency was consistent with the behaviour to inhibition, for it was necessary to allow a single ipsilateral break-shock a definite lead on the excitatory stimulus in order completely to suppress reflex contraction. The single-shock crossed extensor reflex may, therefore, exceptionally possess a latent period of the same order as the knee-jerk or its ipsilateral

inhibition [Jolly, 1910; Ballif, Fulton and Liddell, 1925] and the spinal flexion-reflex [Eccles and Sherrington, 1931*a*].

The spinal crossed extensor response appears to have a latency no longer on the average than the latent period of the crossed extensor reflex in the decerebrate preparation from a single stimulus [Forbes and Cattell, 1924; Pi-Suñer and Fulton, 1929] or even from tetanic stimulation [Fulton, 1926, p. 303; Eccles and Granit, 1929; Ranson, Hinsey and Taylor, 1929].

A period of 60–100 σ , of which not more than a tenth part can be occupied by peripheral conduction, sometimes elapses between the single stimulus and the onset of contraction in the contralateral *soleus*. A latent period of this length calls to mind rebound contraction [Sherrington, 1913], but the frequent brief latencies encountered are a guarantee that the crossed response is a direct excitation. A long latent period is sometimes attached to a vigorous and highly repetitive central discharge. If the long latent period were required for summation sufficient to bring the first motoneurons to the point of discharge, it is unlikely that more than a feeble and single discharge would result. Impulses may reach the anterior horn cells early in the latent period in amounts adequate for discharge, but are suppressed by inhibition. In either case, impulses must arrive at the anterior horn cells as late as $\frac{1}{10}$ second after application of the stimulus. The temporal dispersion of the afferent volley traversing the spinal cord must therefore be great. The extreme variability in latency characteristic of this reflex is evidence that latency is conditioned by some functional and variable (? summative) process and not by fixed delays at synapses, or slower fibre conduction, or some other feature of reflex conduction depending on the nature of the path across the spinal cord.

(b) *Magnitude of reflex response.*

A small proportion of animals examined were totally impervious to a single contralateral excitatory afferent volley, an experience shared by those who have studied spinal and decerebrate preparations [Sherrington, 1910; Forbes and Cattell, 1924; Pi-Suñer and Fulton, 1929]. In other preparations the reflex was irregular and fugitive. There remained, however, a substantial percentage of animals in which the reflex was both strong and regular, though even in favourable preparations considerable variation in the reflexes from identical stimuli has been the rule. The maximum tension developed in *soleus* by a single shock to a contralateral afferent nerve for the individual chronic spinal animal ranges between 50 and 600 g.

Particular interest attaches to the single-shock form of a given reflex because of the opportunity it affords for judging the character of the central discharge. Thus, a single, break-shock, ipsilateral flexion reflex of *tibialis anticus* may exceed in tension the maximal motor twitch of the same muscle [Sherrington, 1921]. This may hold even though a maximal tetanic reflex from the same afferent nerve engages only a fraction of the total motor units making up the muscle [Cooper, Denny-Brown and Sherrington, 1926]. The single-shock crossed extensor spinal reflex can, like the ipsilateral flexion reflex, exceed in contraction tension the maximal twitch of the same muscle recorded immediately afterwards at

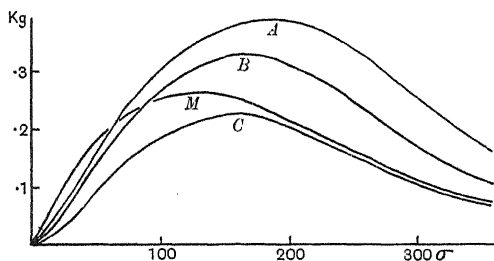


Fig. 1.

Fig. 1. Comparison of single-shock crossed extensor reflexes with the maximal motor twitch (*M*). Chronic spinal cat, 28 days. Deafferented soleus muscle. *A*, *B*, *C*, reflexes at 13, 15, 17 cm. coil distance; *M* at 20 cm. from uncut motor nerve. Weight of muscle 1.77 g.

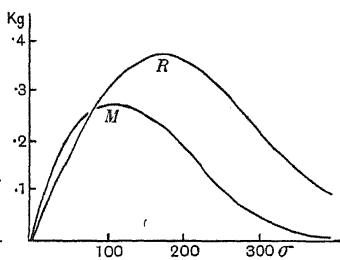


Fig. 2.

Fig. 2. Same. 38-day cat, undeafferented muscle. *M* is motor twitch at 27 cm. (max.), *R* is reflex at 17 cm.

the same initial tension, etc. Experiments of this type are shown by tracings in Figs. 1 and 2. Such superiority is evidence that the central discharge cannot consist merely of a single efferent volley even if, which is unlikely, all motor units composing *soleus* are involved. On the contrary, some motor units exhibit more than one centrifugal volley and by the resulting peripheral summation enable the tension of the reflex to be greater than a motor twitch.

(c) Duration.

When, as is usually true of the single-shock crossed extensor reflex, the tension developed does not attain that of the motor twitch, there is still another feature of the myogram which indicates the character of the central discharge, namely, the duration of contraction. The shortest duration of the single-shock reflex contraction was 81.7σ, as measured by

the "contraction time," *i.e.* the interval from the first sign of electrical response to the point of maximum tension [Cooper and Eccles, 1930]. Contraction times of the order of the motor twitch, *i.e.* 80 to 120 σ , do occur, but are not the rule. In general the reflex contraction time falls within the range 140–200 σ . A variant showing after-discharge of proprioceptive origin is a regular twitch-like contraction to which is appended at some point in relaxation a long and sometimes clonic contraction. Prolongation of duration may mean that the muscle receives a single, but temporally dispersed, efferent volley; since enduring contraction from asynchronous firing occurs at the expense of tension, the combination of ample contraction with long duration points to repetitive central discharge. The single-shock reflexes with durations closely similar to the motor twitch, and also of less tension, are taken to be single volley reflexes such as were commonly encountered in ipsilateral flexion by Eccles and Sherrington [1931a] and by them termed "reflex twitches."

The action current of these crossed extensor "reflex twitches" is not dissimilar qualitatively to that of the motor twitch. It consists of a single initial excursion not followed by the delayed excursions which accompany more prolonged reflexes. In the latter the string galvanometer typically shows activity for a period of many sigmata, and the initiation of mechanical response is often accompanied by only slightly greater excursion, which is always far smaller than a motor twitch action current. Judging from the action current, this reflex is more synchronized in the spinal preparation than in the decerebrate as described by Forbes and Cattell [1924].

Pi-Suñer and Fulton [1929] have recorded decerebrate single-shock crossed extensor reflexes in deafferented vastocruureus with a duration three or four times the motor twitch duration. This degree of prolongation seems never to be approached by the spinal reflex of *soleus* whether normal or deafferented. Observations of Sherrington [1910] and Graham Brown [1911] on tetanic crossed reflexes have shown that after-discharge is curtailed in the spinal form. The crossed extensor "reflex twitch" seems peculiar to the spinal animal. Repetitive central discharge is not an invariable feature of the single-shock spinal crossed reflex. The highly repetitive central discharge of decerebrate crossed extensor reflexes must, therefore, receive explanation on grounds other than features of reflex conduction across the cord as seen in the basic spinal mechanism.

(d) Behaviour to single-shock inhibition.

The elicitation of a reflex response in an extensor muscle by a single unrepeatd contralateral stimulus permits observations on the interaction of inhibition and excitation under some experimental conditions hitherto untried.

The reflex has certain advantages for the investigation of the time relations of the central inhibitory state: first, the central effects of both excitatory and inhibitory stimuli are uncomplicated by summation with inhibition or excitation from brain nuclei; second, the opportunity for repercuSSION of inhibitory impulses within the nervous system is limited; third, the inhibitory path, being ipsilateral, is short; fourth, proprioceptive excitation at initial tensions used is, if present, subliminal. For these reasons the central inhibitory state may be thought of as running its course relatively uninfluenced by excitatory impulses until brought into algebraic summation with a contralateral afferent volley.

By sampling the excitability to a single contralateral break-shock (*E*) at a succession of intervals before and after delivery of a single-shock inhibitory stimulus (*I*) to an ipsilateral nerve, it is possible to follow the changes in size of the reflex response corresponding with the interval of separation of the two shocks. Examples of such experiments are given in Figs. 3 and 4.

In the graphs the ordinates express the reflex contraction tension developed when the contralateral, *i.e.* excitatory, stimulus follows (*I-E* interval, + values to right) and precedes (*E-I* interval, - values to left) an ipsilateral inhibitory shock. The intervals *I-E* and *E-I* are shown by distance on the abscissa line. Owing to the variability in magnitude of the control *E* response our practice was to record alternately with the inhibited reflex an uninhibited control reflex. These are entered over their fellow inhibited reflex. The vertical distance between the average of the controls and the line joining the inhibited reflex points is an index of inhibition existing at any given *E-I* or *I-E* interval.

In the first limb of the curves the excitatory preceded the inhibitory stimulus because, owing to latency, the inhibitory shock may fall after the excitatory and yet be effective [Sherrington, 1925]. The first critical point is the *E-I* interval at which the central process underlying the contraction ascent has just been completed before the *I* volley reaches the centre or develops its effect. As the *E* stimulus is allowed less and less lead over *I*, the reflex contraction suffers progressively a loss of tension. A second critical interval is that at which an *I* stimulus falls just sufficiently early to block all reflex discharge. The stimulus, maximal in strength, is also just optimal in point of time relation to the excitatory stimulus. As the *I* shock is brought nearer to and made to

precede the *E* stimulus there is usually a steadily maintained deep inhibition, after which the value of the contraction response commences to rise, indicating the omission of the earlier excited motoneurons from inhibition. Now if the *E* reflex be initiated at longer and longer intervals after *I*, it steadily increases in tension. Often just before normal size is gained the curve of recovery flattens.

The position of the four points of inflection varies greatly in different experiments. The inhibition from a single shock may persist as long as

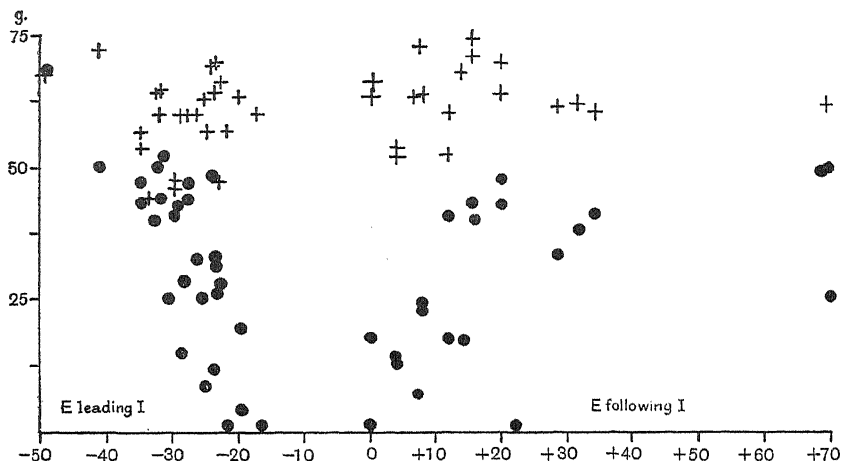


Fig. 3. Inhibition of the single-shock crossed extensor reflex by a single ipsilateral stimuli falling at intervals preceding (+) and following (-) the *E* stimulus. The ordinates give contraction tension. Zero on the abscissa line represents the application of the inhibitory stimulus and the position of a dot on the abscissa scale gives the time of application of the excitatory stimulus. A dot is an inhibited reflex, a cross an uninhibited control reflex recorded immediately after one of the inhibited reflexes beneath it. *E* stimulus 20 cm. coil distance, *I* stimulus 24 cm. Average latency of control reflexes is 29.5σ.

465σ, the longest interval open to exploration with the Lucas pendulum; or more rarely, it may run through its course in a tenth of this time. Curves of almost all intermediate grades have been mapped out, but usually the recovery of excitability has been slow. A long enduring inhibitory after-action may represent an equally long enduring central inhibitory state, *per se*, or merely a rapidly effected destruction of some facilitative background essential to the reflex, which is but slowly reconstituted [Sherrington, 1929]. Against the latter possibility is that typically long persisting inhibitory after-discharge has been demonstrated after deafferentation of the muscle.

In addition to earlier observations several recent studies [Ballif, Fulton and Liddell, 1925; Samojloff and Kisselew, 1927; Eccles and Sherrington, 1931*b*, 1931*c*; Bremer, 1931] are in accord that a single inhibitory volley has characteristically a prolonged after-action when excitatory destruction or masking by inhibition is not an obvious factor. The same conclusion can be extended to single-shock inhibition

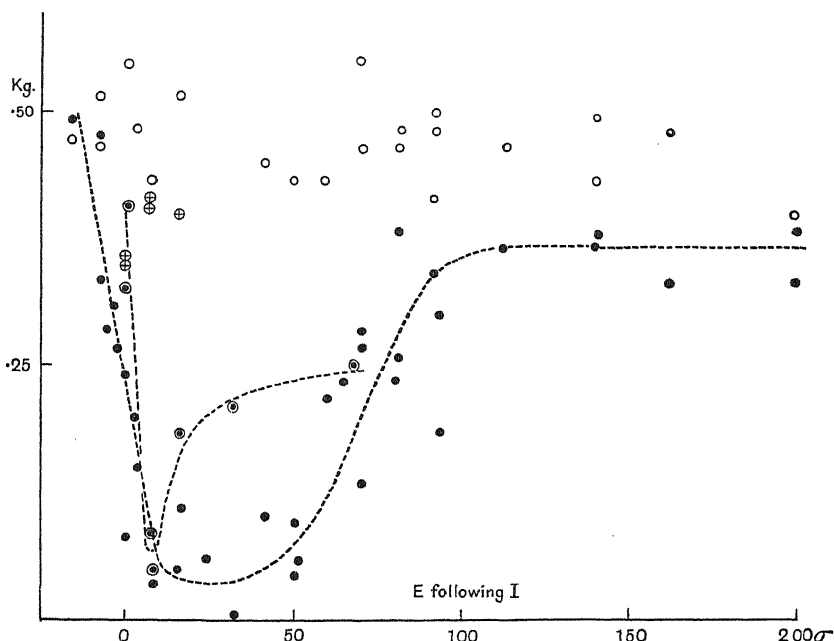


Fig. 4. Same as Fig. 3. Circles represent tension of control reflexes paired with underlying dot. E is 20, I is 25 cm. coil distance. Crossed and dotted circles are another series from the same preparation with E at 20 and I at 25 cm.

of this single-shock crossed extensor reflex. This type of inhibition is not, however, necessarily prolonged. There are some instances of brief-lasting inhibition in our results. The briefest inhibition recorded by us (Fig. 3) reached an optimum in about 15σ after the first sign of inhibition was manifest; after a maximal plateau of 10σ the decline of inhibition was largely over in another 25σ . Inhibition on some motoneurons was as short as 25σ , and 50σ is the longest time for which inhibition of any motoneuron can have persisted. In one series of observations in Fig. 4 the cycle is complete within 100σ . But since these preparations were undifferentiated the factor determining the rate of disappearance of

inhibition may still be the amount of subliminal excitation which the inhibitory volley meets.

The greatest E - I interval for occurrence of complete inhibition is determined by the relative latency of E and I reflexes. The earliest inhibitory impulses must arrive not later than the time at which the excitatory impulses reach the motoneurons in quantities adequate for discharge, and so the interval will be the difference between central time of effective excitation and inhibition, the lengths of the peripheral paths being equal. It should be possible, therefore, to calculate the latency of inhibition from the length of this interval and the latent period of the excitatory reflex.

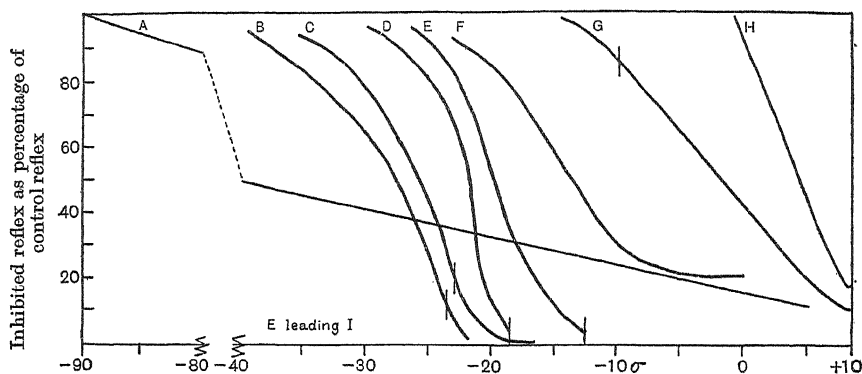


Fig. 5. Curves from different experiments, like Figs. 3 and 4, showing the relation of single-shock crossed extensor tension to E - I interval. The ordinates give the tension of inhibited reflexes on which each curve is based as a percentage of the average tension of the accompanying control reflexes. The vertical lines show the average latency of the uninhibited reflexes less peripheral conduction time. For significance see text.

In Fig. 5 vertical lines are drawn across the E - I curves at intervals from zero found by deducting from the latent period of the uninhibited reflexes the time required for peripheral conduction estimated at 6.5σ [Eccles and Sherrington, 1931*a*]. The distance of this line from the intersection of curve and base line is a measure of central inhibitory time¹. In four of the curves the inhibition is almost complete at the interval indicated by the vertical line, and the base line would always be reached in a few stigmata, thus indicating the shortness of central inhibitory time. Since in this time are included both central conduction time and any delay for summation to reach an effective level, the latter must be very short.

The inhibitory stimulus was maximally effective in G , Fig. 5, only when it was given a lead of 10σ over the E shock, despite that in all other experiments I was effective when following the E stimulus. Even with regard to the short latent period of the E reflex,

¹ If d is the difference in central excitatory time (e) and central inhibitory time (i), i.e. $d = e - i$, then $d - e = i$; d and e can both be determined experimentally.

16.4 σ , it is surprising that the latency of inhibition seems actually longer. The central inhibitory time is calculated to be 19.9 σ . While the central discharge was undoubtedly highly repetitive, as judged by tension, duration and action current, an *I* stimulus affected these little provided the excitatory volley was given 10–20 σ lead. The central excitatory state underlying this reflex was presumably inhibitable only after a long period of inhibitory summation. Similarly, in the experiment for curve *F*, Fig. 5, in which inhibition was not maximal at any interval, the central inhibitory time was also much longer than in other experiments, being about 40 σ . The central time of an ipsilateral inhibition in quantities maximal for a given excitatory reflex may, therefore, be a few sigma or as long as 40 σ . Asynchronous inhibitory after-discharge would provide a basis for long central time, the figures given being the point in the inhibitory after-discharge at which sufficient impulses arrive at the anterior horn cell for complete inhibition.

The dependence of reflex tension on the length of the *E-I* interval, as shown in Figs. 3, 4, and 5, suggests a gradual encroachment of inhibition, when given earlier and earlier inception, on the period of central discharge, gradually eroding it away until complete extinction of the reflex is effected. If this is true, those reflexes which are initiated close before an inhibitory stimulus should suffer a shortening of duration as well as loss of tension as compared with an excitatory reflex which had been given an earlier start before the inhibitory stimulus. This curtailment of the contraction response does occur when the excitatory reflex is repetitive, as is shown by Figs. 6 and 7*a*. The lessening of tension and of contraction time run roughly parallel up to a certain point, after which there is no further curtailment, though the tension continues to decrease. This point approximates to the contraction time and maximal tension of the motor twitch. In such experiments the temporal dispersion of the repetitive central discharge is in part responsible for the greater effectiveness of an early than a later inhibitory volley.

The lessening of contraction tension brought about by approaching the *I* to a preceding *E* stimulus is in many experiments not accompanied by significant alteration in duration of reflex response. This is illustrated by superimposed tracings in Fig. 7. When the reflex behaves to inhibition in this fashion a small decrease in the lead of *E* over *I* stimulus often suffices to change inhibition from a minimal to a maximal level, which contrasts with the long span of the *E-I* curve in Figs. 6 and 5*A*. Further, the excitatory reflex is found to be of short duration and usually of small tension. The behaviour to inhibition confirms the evidence given above for the existence of single centrifugal volley crossed extensor reflexes or "reflex twitches." Though a single volley alone is produced, all or nearly all of the motoneurones may participate (Fig. 7).

There remains to ask the reason for the dependence of reflex tension on the length of the *E-I* interval when the reflex is of the "twitch" type.

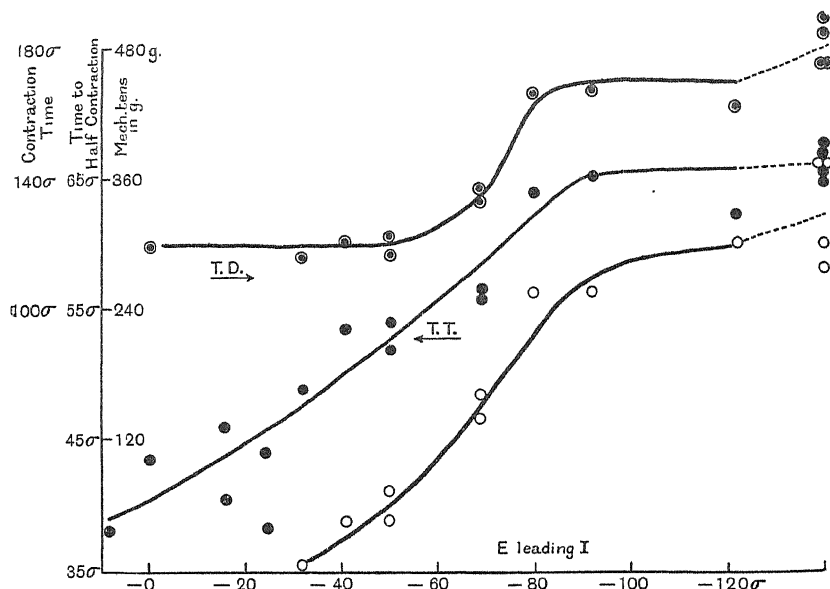


Fig. 6. The curves show the effect of ipsilateral inhibition on tension of single-shock crossed extensor reflex (dots), the time to half contraction (circles) and the time to point of maximal contraction (dotted circles). *T.T.* indicates tension, and *T.D.* the contraction time of the maximal motor nerve twitch of the recording muscle.

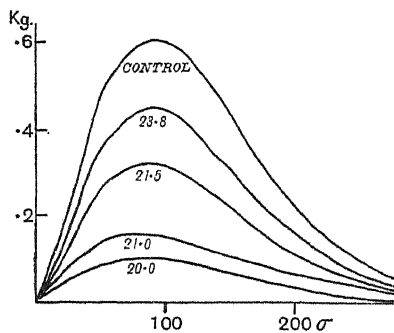


Fig. 7.

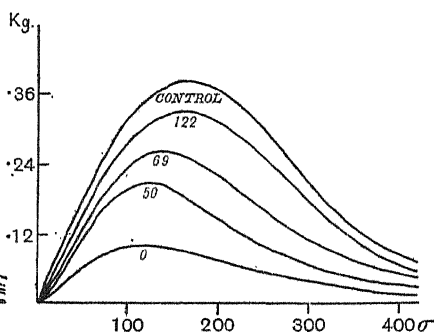


Fig. 7a.

Fig. 7. Superimposed tracings of single-shock crossed extensor reflexes inhibited to different extents by a single ipsilateral stimulus. The number attached to each curve is the interval in σ by which the *E* preceded the *I* stimulus. Shortness of contraction time (despite a strong tension development) is associated with absence of shortening by inhibition. *E* stimulus, 9 cm. and *I* stimulus 17 cm.

Fig. 7a. Same treatment of another experiment. *E* 17 cm. and *I* 11.5 cm. Maximum motor twitch was 200 g. Excess of contraction time over motor twitch is associated with a shortening of reflex discharge as the *E-I* interval is lessened.

In these reflexes temporal dispersion is confined to asynchrony of the single efferent volley. Asynchrony of the inhibitory volley in its arrival at the point of interaction with excitation probably combines with the temporal dispersion of the excitatory volley to make an early *I* stimulus more effective than a late one. When the excitatory reflex is of the "reflex-twitch" type, the slope of the *E-I* curve is probably largely fixed by the character of the inhibitory after-discharge. Thus, according to our observations, the *E-I* curve is determined by the character of both the excitatory and inhibitory volley.

The inhibition of single-shock crossed extensor reflexes differs from the contralateral inhibition of the ipsilateral flexor reflex as described by Eccles and Sherrington [1931 c] in these ways: complete inhibition of the single-shock crossed extensor reflex by a single inhibitory shock is more easily obtained; the span of the *E-I* curve is typically less when the excitatory reflex is of the "reflex twitch" type; the after-action of a single ipsilateral inhibitory stimulus is often briefer. These differences are consistent with the paths which inhibitory and excitatory volleys follow in the two kinds of experiment. After allowing for differences in the inhibition curves imposed by difference in the excitatory reflex, some of the same broad differences appear to exist between ipsilateral and contralateral inhibition that exist between ipsilateral and crossed excitation.

CONCLUSIONS.

1. Single afferent volleys are effective stimuli to contralateral extension in the chronic spinal cat, despite the "inertia" of conduction across the spinal cord and the depression of extensor reflexes in the spinal condition.

2. Single-shock crossed extensor reflexes are typically of short latency ($20-35\sigma$), though the latent period may be as short as 8.7σ or as long as 100σ .

3. One variety is of small tension (60–200 g.), of brief duration and myographic form not greatly different from the motor twitch, and is probably a single, asynchronous efferent-volley reflex. In a second variety, a repetitive central discharge is shown by a greatly prolonged mechanical and electrical response, and more rarely, by excess of reflex over maximal motor twitch tension.

4. The degree of inhibition of the reflex by an ipsilateral single shock depends on the interval separating the two stimuli. The level of inhibition increases rapidly as an ipsilateral stimulus is approached in time to a

preceding contralateral shock until an optimal interval is reached. Despite its ipsilateral path, inhibition wanes slowly when sampled at intervals by a following excitatory stimulus. Inhibition can, however, largely subside in a period as short as 25σ .

5. Inhibition can be used analytically to distinguish between reflexes of single asynchronous and repetitive central discharge.

In some experiments we had the pleasure of the collaboration of Prof. A. Forbes of the Harvard Medical School. We wish to express our gratitude to him and to Prof. Sherrington for much helpful advice. To the latter we owe the suggestion of making the spinal single-shock contralateral reflex of *soleus* in the spinal mammalian preparation the subject of investigation.

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RESPIRATION OF ISOLATED GILL TISSUE OF THE EEL.

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(From the Physiological Laboratory, Cambridge.)

INTRODUCTION.

VARIOUS attempts have been made to show that osmotic regulation in marine and fresh-water animals is accompanied by increased respiratory exchange. The evidence is almost entirely presumptive; the respiration of a tissue is measured under conditions in which activity is assumed to occur without any proof of its occurrence; still further, from a direct correlation, the respiration of an entire animal is measured under two sets of conditions involving respectively quiescence and activity of the osmo-regulatory mechanism.

The results are open to some obvious objections. The animals may be more active, mechanically, under one set of conditions [Beadle, 1931; A. V. Hill, 1931, p. 69]—a criticism whose force is shown clearly by the experiments of Kreps [1929] on the respiration, in water of different salinities, of *Balanus crenatus*. Kreps found that cessation of motility was accompanied by a decrease of respiration to 1/3 or 1/4 of its normal value, while changes which could be attributed directly to changing salinity were of a much smaller order. Again, total respiration of an entire animal may be subject to complex regulatory influences which may mask the behaviour of any one organ. Schlieper has upheld the view that osmo-regulatory activity is accompanied by increased respiration of the animal and he has supported it by observations on the shore crab, *Carcinus maenas* [1929, 1930], and on *Gammarus locusta* [1931], and by the experiments of Tarussov [1927] on *Nereis diversicolor*³. In the first case the conditions for osmo-regulatory activity are well defined, but the question of muscular activity is a doubtful one; in the case of *Gammarus* there is no information concerning the behaviour of the body

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³ Dr Schlieper informs us that he has recently obtained similar evidence in the case of some other forms, particularly *Eriocheir sinensis*.

fluids when the animal is in different environments, and therefore no experimental evidence in favour of Schlieper's interpretation. The evidence in the case of *Nereis diversicolor* derives some validity from Beadle's [1931] experiments, in which it was found that osmotic regulation occurs although it is absent in the closely related form, *Nereis cultrifera*. Other experiments of this kind are those of Beadle [1931] on *Gunda ulvæ*, the osmotic properties of which had been studied by Pantin [1931] and Weil and Pantin [1931], and the observations of Hayes [1930] on *Paramœcium*.

Where the osmo-regulatory process is probably more complex, as in the fishes, observations on the intact animals are still more difficult to interpret. Raffy and Fontaine [1930] reported that "civelles" adapted to fresh water showed a greater oxygen consumption than the same animals adapted to sea water, although a prolonged sojourn in fresh water presumably causes cessation of gill secretory activity. Keys [1931 a], on the other hand, found that the respiration of *Fundulus parvipinnis* is depressed in fresh water.

Schlieper [1929] measured the respiration of the isolated gill of the mussel, *Mytilus edulis*, and found that the gas exchange is greater in diluted media than in pure sea water. Here again, however, the observations are for the present of little significance, because there is no evidence that the gills of *Mytilus* play any rôle in osmotic regulation; it is not even known that osmotic regulation occurs in this animal.

The present experiments were undertaken in view of the possibility that the secretion of chloride, which is manifested by eel gills under certain conditions [Keys, 1931 c], might also occur in the extirpated gills under similar conditions. It was shown in Keys's experiments that the amount of secretory activity of the gills is determined by the internal concentration of chloride, so that no secretion is observed when the internal medium has a freezing-point depression of less than 0.50° C.; as the internal concentration is increased above this point the secretion is initiated and further small increases in the internal concentration provoke progressively larger increases in the secretion activity. These results have been confirmed by Krogh and Schlieper (unpublished experiments), who showed that the external concentration is not of itself a major factor in the phenomenon.

The conditions of internal concentration which maintain the gills active or passive as regards secretion can be brought about almost as readily with the gills extirpated as *in situ*. The perfusion technique worked out by Keys [1931 b] made it possible to fill the gills with fluid of any

desired concentration. When these gills are extirpated and suspended in an appropriate solution, diffusion and passive osmotic interchange between the internal and external solutions will tend to keep the internal concentration high or low, depending on the concentration of the external solution.

If the gills on one side of an eel are filled with Ringer's solution of $\Delta = 0.70^{\circ}\text{C}$., extirpated and suspended in a balanced medium of high concentration, it is not too much to expect that they will be more active in secretion than the gills from the other side of the same animal, which are similarly perfused with a Ringer's solution of $\Delta = 0.50^{\circ}\text{C}$. and suspended in a medium of low concentration. With preparations of this kind transferred to microrespirometers, it should be possible to obtain presumptive evidence for or against the utilization of oxygen in the branchial secretory process. It should be noted that the great exposure of surface in the gills makes them peculiarly suitable for respiration measurements.

EXPERIMENTAL.

The following account deals with the general procedure adopted in most of the experiments.

The eels (*Anguilla vulgaris*) used were kept in the laboratory in a tank of running tap water; some of these were transferred to sea water and allowed a few days for acclimatization before being used. The gills were perfused from the ventral aorta [Bateman and Keys, 1932], the cannula being connected by a two-way tap to two reservoirs containing respectively "concentrated" and "dilute" Ringer's solutions [prepared according to Keys, 1931 b] or different dilutions of sea water. The "concentrated" sea water had a freezing-point depression of 0.70°C . and the "concentrated" Ringer's solution a Δ of 0.72°C . The "dilute" sea water and the "dilute" Ringer's solution had freezing-point depressions of 0.50°C . and 0.55°C . respectively. In a few cases the animals were anaesthetized with amytal [Keys and Wells, 1930], but the amytal was washed out rapidly by the subsequent perfusion.

After perfusion with a given solution for some minutes, the gills on one side were exposed by cutting open the operculum and the afferent and efferent ends of the gill bars on this side were clamped off. At this time the reservoir tap was turned so as to perfuse the opposite side with the second solution. The first set of gills was excised by cutting away from the clamps and was suspended in the appropriate medium. After 10–15 minutes the second set of gills was excised similarly. The individual

gill bars were carefully separated and weighed in Ringer's solution after gentle blotting with clean filter paper. The fourth (posterior) bar on each side was discarded on account of its small size. It should be mentioned that in all these operations the gill filaments themselves were touched only in the process of removing excess liquid incidental to weighing, and, save at the extreme ends of the bar, the gill filaments suffered no manipulative damage whatever.

The gills perfused with the dilute and concentrated Ringer's solutions were now transferred to Barcroft manometers containing 3 c.c. 1/9 and 5/6 sea water respectively, and 0.5 c.c. 40 p.c. KOH. After a period of 20 minutes for temperature and gas equilibration, the taps were closed to the air and manometer readings taken at 15-minute intervals for several hours, bath temperature and barometric pressure being recorded. When the measurements were completed the gills were weighed after gentle blotting, re-weighed after being heavily blotted to remove all adherent moisture, and finally the gill filaments were dissected away from the cartilaginous bar and the weight of the latter obtained. This dissection could be made quite cleanly and the proportion of relatively inert cartilage and non-filament tissue in the gill was obtained sufficiently accurately for an adequate correction to be made in computing the respiration of the gill-filament tissue proper. The respiration of the "cartilage" alone was determined in several experiments, and a mean value used in the application of this correction. The constants for the Barcroft manometers were corrected in each experiment for the different volumes of liquid and tissue used.

PROPORTION OF GILL RESPIRATION DUE TO "CARTILAGE."

The figures for "cartilage" respiration are given in Table I. The values for each hour of experiment are the means of the four 15-minute measurements. The mean values, 54.3 c.mm. O₂ per g. per hour for the first hour and 49.4 for the second, were used in all calculations. The

TABLE I. Respiration of gill cartilage at 15° C.
Mean respiration in c.mm. O₂ at N.T.P.
per g. per hour

Exp.	Mean respiration in c.mm. O ₂ at N.T.P. per g. per hour	
	First hour	Second hour
C 1	65.8	52.6
C 2	74.4	86.5
C 3	45.5	30.3
C 4	45.7	40.1
C 5	39.9	37.3
Means	54.3	49.4

rather wide variation in the individual values is probably due to incomplete removal of filament tissue, making the lower figures more probably correct. But the corrections involved are small enough for the mean value of all experiments to be used without serious error.

The proportion of the measured weight of gill bars due to "cartilage" was found usually in each individual case, but, as this determination was sometimes neglected, a mean value had to be obtained from all the figures available. These seem to be worth presenting, since they illustrate the cleanness with which the dissection of cartilage could be done (Table II). The mean value, 48 p.c., was used in calculation.

TABLE II. Proportion of cartilage in gill bar.

Series No.	No. of determinations	Mean weight of cartilage as p.c. of total blotted dry weight
P 1	6	48
P 2	6	47
P 3	6	49
P 4	6	42
P 5	2	44
P 6	2	51
P 7	4	43
P 8	4	50
P 9	2	54

The experimental procedure and method of calculating results may now be made clearer by a detailed typical protocol. The remaining data will be presented more briefly by graphs and summarizing tables.

Protocol of Exp. 2. 25. iii. 1932.

Eel from fresh water after 2 days in laboratory. Operation begun 12.17 p.m., perfusion begun 12.20 p.m. Operation complete 12.22 p.m. Internal perfusion media: 1/3 sea water from start till 12.53 p.m., 1/4 sea water till 1.5 p.m. External medium: tap water. Gill from right side dissected out at 12.52 p.m. and transferred temporarily to 1/3 sea water. Left side out at 1.5 p.m. and transferred to 1/4 sea water. Placed in respiration chambers at 1.15 p.m., each apparatus containing 3.0 c.c. external medium and 0.5 c.c. 20 p.c. KOH. The manometer readings were continued for 4.5 hours, then the tissue was weighed. The details of external and internal media, weights of tissues, and manometers are given in Table III, while Table IV shows the detailed figures for one manometer, together with the essential stages in the calculation.

The constant k for manometer 75, determined at 15.5° C. and 763 mm. and containing 0.1 c.c. liquid, was 3.08, or 2.90 at N.T.P. For this experiment, with cups containing 3.5 c.c. liquid + 0.177 g. tissue at 14.3° and 760 mm., k becomes 2.43. Then the rate of O_2 uptake is kx (see Table IV). This is due partly to respiration of gill filaments and partly to cartilage. Correction for the latter is applied by subtracting from kx the product (weight of cartilage) \times (mean respiration rate of cartilage) = B , or $0.085 \times 51.8 = 4.6$ (see Tables I and III). The true respiration of gill tissue is then this difference divided by the weight of gill tissue (M). In Table V the results of the calculation are given for all six manometers.

TABLE III. Weights of tissue, etc., in Exp. 2.

1. Apparatus No.	75	76	78	114	116'	116
2. Internal medium	Dilute Ringer's solution			Conc. Ringer's solution		
3. External medium	1/9 sea water			5/6 sea water		
4. Weight of gills at end of exp.: g.	0.177	0.182	0.121	0.158	0.161	0.134
5. Weights of gill bars calc. as $0.48 \times (4)$	0.085	0.087	0.058	0.076	0.077	0.064
6. Weights of gill filament tissue (M)	0.092	0.095	0.063	0.082	0.084	0.070

TABLE IV. Calculation of respiration rates in Exp. 2.

Manometer 75. $k = 2.43$. Weight of gill tissue 0.092 g.
Cartilage correction, $B = 4.6$ throughout.

Time (min.)	Rate of movement of manometer liquid: = x mm./hour	kx c.mm. O_2 per g. per hour	$kx - B$	$\frac{kx - B}{M}$
0-15	16.8	40.8	36.2	394
15-30	14.0	34.0	29.4	320
30-45	11.2	27.2	22.6	246
45-62	11.3	27.5	22.9	249
62-90	11.4	27.7	23.1	251
90-105	9.6	23.3	18.7	203
105-120	11.6	28.2	23.6	256
120-135	10.8	26.3	21.7	236
135-150	10.0	24.3	19.7	214
150-165	12.0	29.2	24.6	267
165-180	10.0	24.3	19.7	214
180-215	9.9	24.3	19.7	214
215-245	10.0	24.3	19.7	214
245-275	9.2	23.3	18.7	203

TABLE V. Final results for Exp. 2.

Respiration rates for gill tissue in c.mm. O_2 per g. per hour at N.T.P. Temperature = $14.3^\circ C$.

Manometer No.	Internal medium "dilute" Ringer's solution. External medium 1/9 s.w.			Internal medium "concentrated" Ringer's solution. External medium 5/6 s.w.		
	75	76	78	114	116'	116
Time (min.)						
0-15	394	217	351	458	392	456
15-30	320	285	276	473	408	433
30-45	246	252	320	416	424	261
45-62	249	228	198	358	286	224
62-90	251	194	220	365	321	252
90-105	203	252	232	372	240	188
105-120	256	194	246	401	348	247
120-135	236	194	246	358	339	321
135-150	214	182	203	386	332	203
150-165	267	205	173	372	348	203
165-180	214	194	246	328	332	247
180-215	214	235	198	379	336	228
215-245	214	171	195	328	324	166
245-275	203	171	218	328	282	240

s.w. = sea water.

RESULTS.

The figures obtained in the detailed protocol, which are plotted in Fig. 1, are typical. In all other experiments of the same kind the respiration was definitely greater when the liquid bathing the gills was sea water or 5/6 sea water than when it was tap water or 1/9 sea water. Individual manometers very occasionally, as in the experiment quoted, gave irregular results, which could as a rule be traced to an imperfectly greased tap. In Fig. 2 the results of a similar experiment are plotted, the ordinate here being total O₂ uptake per g. gill tissue instead of the rate which is plotted in Fig. 1. This shows the main effect rather more strikingly.

TABLE VI. Respiration of isolated gills in 1/9 s.w. and in 5/6 s.w.

Experi- mental series	Internal media	Mean respiration rate in c.mm. O ₂ per g. per hour				Incre- ment, rate in 5/6 s.w. minus rate in 1/9 s.w.	Incre- ment as p.c. of values in 1/9 s.w.
		1/9 s.w.	Mean of series	5/6 s.w.	Mean of series		
2	"Dilute" R in 1/9 s.w.	274.0	256.3	406.1	348.7	92.4	36
	"Conc." R in 5/6 s.w.	231.7		345.6			
		263.3		294.4			
3	1/4 s.w. in 1/9 s.w.	68.7	77.3	120.0	116.2	38.9	50
	1/3 s.w. in 5/6 s.w.	86.0		112.5			
4	Same as series 3	215.5	211.3	227.0	301.5	90.2	43
		211.5		344.5			
		207.0		333.0			
10	Same as series 2	286.0	288.1	308.7	323.1	35.0	12
		289.0		337.5			
		289.2					
11	Same as series 2, plus glucose and urea	269.5	208.6	387.7	344.4	135.8	66
		204.5		367.5			
		151.9		278.0			
16	Same as series 2	220.6	220.6	386.6	386.6	148.0	67
						Mean of all	46 p.c.

Each value given is the mean of from 6 to 12 determinations; a total of 234 determinations is summarized in this table. R=Ringer's solution.

s.w. = sea water.

The remaining experiments are summarized in Table VI, in which the mean rates of respiration are given for each manometer used, over a period of 2 hours. In column 7 the differences between the mean rates in the two media are given for each experiment, this difference being expressed in column 8 as a percentage of the "normal" respiration in 1/9 sea water. It is clear that there is a consistent, though rather variable, positive difference, amounting on the average to about 46 p.c. of the respiration in 1/9 sea water. The variability is to be expected in a

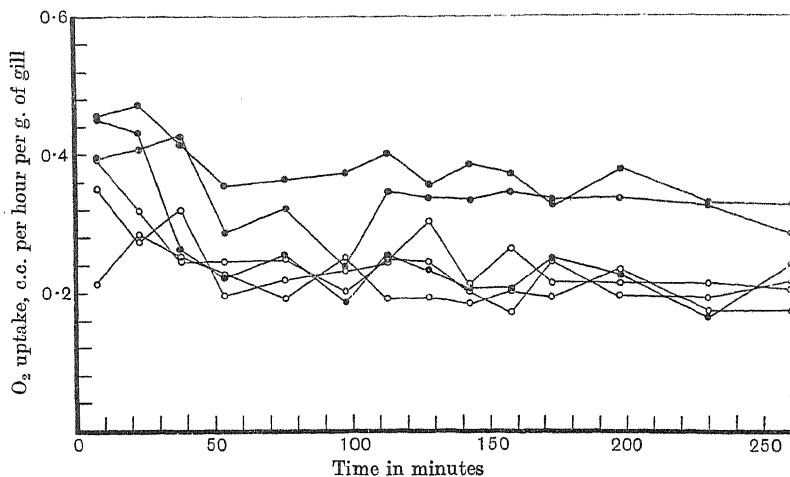


Fig. 1. Effect of salt concentration on the respiration of extirpated gill tissue. Rates of respiration of six gills from a single eel. Solid circles, gills perfused with Ringer's solution $\Delta = 0.70^\circ$ and suspended in $5/6$ sea water. Open circles, perfused with Ringer's solution $\Delta = 0.50^\circ$ and suspended in $1/9$ sea water. Temp. 14.3° .

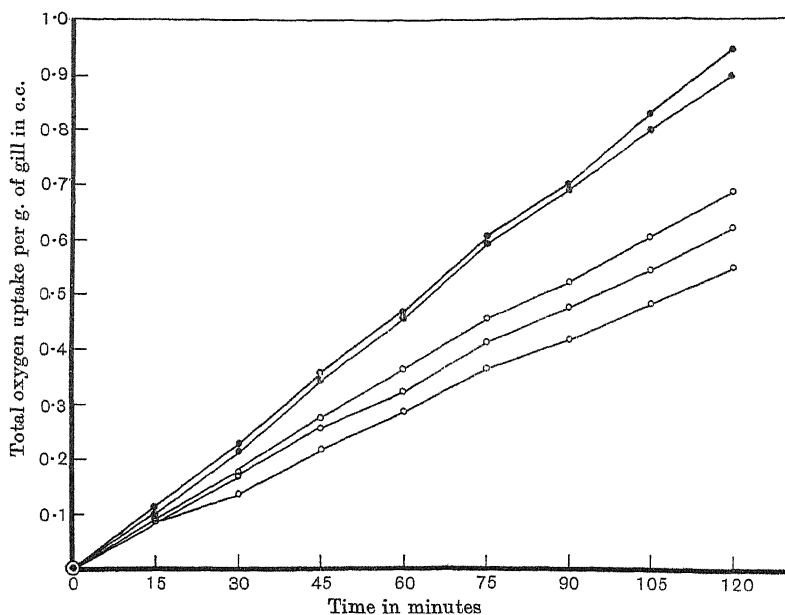


Fig. 2. Effect of salt concentration on the respiration of extirpated gill tissue. Total respiration of five gills from a single eel. Solid and open circles as in Fig. 1. Temp. 14.6° .

tissue subjected to such drastic manipulation before the performance of the experiment. There was generally a tendency for the respiration to decrease somewhat during the course of the experiment; this may be seen in Fig. 1, which is typical of the behaviour observed. The data are not, however, sufficiently extensive to indicate any tendency of the increment due to salt concentration to decrease more rapidly than the total respiration.

The existence of the effect under slightly more physiological conditions was confirmed in a series of experiments on the blood-filled gills. The procedure was as follows: the eel was pithed and the gills exposed with care to avoid serious bleeding. The afferent and efferent ends of the gills were clamped with hæmostats, as in the previous case, and the gills excised with the clamps still in position. Then the individual gill bars were ligatured at both ends, the clamps removed, and the gill bars cut apart. The respiration experiments were carried out as in the other experiments and the results are summarized in Table VII. In Fig. 3

TABLE VII. Respiration of blood-filled gills in dilute and concentrated media.

Ex- peri- mental series	Pre- habi- tat of eel	Ex- ternal solu- tion	Resp. rate	Mean rate	External solution	Resp. rate	Mean rate	Incre- ment, conc. minus dilute rate	Incre- ment, as p.c. of dilute rate
5	s.w.	f.w.	146.0 127.2	136.6	s.w.	290.0 218.5 472.5	327.2	190.6	140
7	f.w.	"	213.5	213.5	"	368.5	368.5	155.0	71
8	"	"	158.2	158.2	"	223.5	223.5	65.3	41
9	"	"	97.0	97.0	"	228.7	228.7	131.7	135
12	"	1/9 s.w.	177.5 128.9	153.2	5/6 s.w.	206.2 244.7	225.4	72.2	47
15	s.w.	"	190.0 187.7	188.8	"	289.0 241.5	265.2	76.4	40

s.w. = sea water; f.w. = fresh water.

Mean of all 79 p.c.

the data of experiments 7, 8 and 9 are plotted. In each of these three experiments a small eel was used, and the whole gill from one side put into one respiration chamber. It appears that, although in a single experiment the respiration was more constant under these conditions than in the perfused gills, the actual O_2 consumption was somewhat smaller and the increase of respiration in sea water even more marked, having a mean value 79 p.c. more than that of the respiration rate in fresh water. For gills from eels acclimatized to sea water the mean is 76 p.c. and for those from fresh water it is 82 p.c.; considering the wide variation in the quantities observed this agreement may be fortuitous.

The most rigid proof of the reality of the phenomenon under discussion would be to show the effects of salt concentration on the respiration of the same portion of tissue. Such an experiment would put a severe strain on the capacity of the extirpated tissue to adjust to the changing concentration of its environment, and it could hardly be expected that

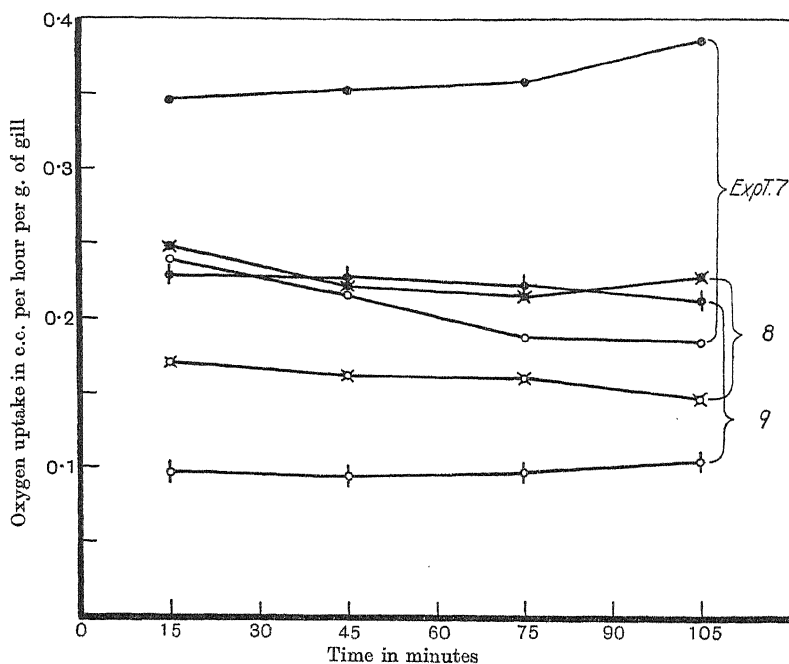


Fig. 3. Effect of salt concentration on the respiration of extirpated blood-filled gills. Three experiments, eels from fresh water. Solid circles, gills suspended in sea water. Open circles, gills suspended in tap water. Temp. 14.0° .

the secretory mechanism would respond perfectly under these circumstances. Experiments, however, showed that under these conditions there is an effect of salt concentration on the oxygen consumption which, although small, is in agreement with the earlier experiments; *i.e.* the oxygen consumption tends to be higher in the more concentrated environment. Table VIII summarizes the results of these experiments in which the respiration of individual gills was measured first in an environment of one concentration and then in an environment of a very different concentration.

TABLE VIII. Effect of change of salt concentration on the respiration of individual gills.

Group A, internal concentration initially $\Delta = 0.70^\circ \text{C}$.

Gill No.	First observations, relative resp. rate in environment $\Delta = 1.7^\circ \text{C}$.	Second observations, relative resp. rate in environment $\Delta = 0.3^\circ \text{C}$.
1	100	82.0
2	100	86.5
3	100	104.0
4	100	81.0
5	100	71.0
6	100	85.5
7	100	92.0
Means	100	86.0

Group B, internal concentration initially $\Delta = 0.48^\circ \text{C}$.

Gill No.	Second observations, relative resp. rate in environment $\Delta = 1.7^\circ \text{C}$.	First observations, relative resp. rate in environment $\Delta = 0.3^\circ \text{C}$.
8	100	94.5
9	100	97.5
10	100	78.0
11	100	91.0
12	100	83.0
13	100	84.5
14	100	86.5
Means	100	87.9

DISCUSSION.

The increase in respiration with increase in the salt content of the environment has been observed regularly under different experimental conditions, and its reality seems certain. Its interpretation in terms of the performance of secretory work by the gills is simple and attractive, but it is necessary to consider the possibility that some other effect of changing salinity may be involved.

The effects observed in the various experiments cannot be ascribed to any difference in the $p\text{H}$ of the solutions. The initial $p\text{H}$ of the internal solutions was always between 7.5 and 7.7, and the $p\text{H}$ of the external solutions was between 7.8 and 8.0; the shift to greater alkalinity due to abstraction of CO_2 in the respiration chambers was practically the same in the various solutions. As to the normality of the tissue respiration observed, we may mention that the effect of cyanide on the gill respiration was quite normal.

In some cases it appears that tissue respiration, even where no secretory activity is known to occur, may be a function of the salt concentration of the environment, but there is no general ground upon which pure osmotic effects on respiration can be predicted. The effect of

hypertonic NaCl solutions in increasing the respiration of fertilized sea-urchin eggs [Warburg, 1909; Meyerhof, 1911], for example, was supposed by Warburg to be a direct effect of NaCl on the respiration, while Schlieper [1931] attributes it to an attempt to prevent, by an active osmotic regulation, the entry of the poisonous NaCl. With plant cells increase of internal osmotic concentration produces no predictable general effect [Smith, 1915-16; Palladin and Sheloumova, 1918; Inman, 1921]. Hayes [1930] found that the respiration of *Paramecium* is increased both in diluted and in concentrated media. Clearly, in these cases Schlieper's explanation of the Warburg sea-urchin eggs experiment cannot be applied. In the present instance, calculation shows it to be quite possible that the decreased respiration in dilute sea water may be to some extent an injury effect due to salt loss to the surroundings; if we reason thus we must conclude that the tissue in 5/6 sea water suffers to an even greater extent from dehydration. The dilute sea water (Δ = about 0.23°C.) is much nearer the normal internal osmotic pressure of the gills (Δ = about $0.6-0.7^{\circ}\text{C.}$) than the concentrated sea water (Δ = about 1.65°C.). Moreover, the observed respiration of gill tissue immersed in 1/3 sea water is rather less than in 5/6 sea water, as is shown in Table IX.

TABLE IX. Respiration of gills in 1/3 s.w. ($\Delta=0.67^{\circ}\text{C.}$).
Respiration rates in c.mm. O_2 per g. per hour.

Exp. series				Mean rate	Mean respiration rate of gills in 5/6 s.w.
A	B	C	D		
427	324	208	237	292	302
407	304	226	264		
411	263	212	246		

s.w. = sea water.

In the attempt to discover whether there is a general effect of salt concentration on the respiration of eel tissues, we have studied the respiration of the thin membranous fins, which, like the gills, are well suited for respiration measurements and are subjected to great variation in the external concentration in the normal life cycle of the animal. The technique was similar to that used in the gill experiments; the fins were extirpated and the respiration of the fin from one side of the eel was measured in 1/9 sea water, while the respiration of the corresponding fin from the other side was measured in 5/6 sea water. In each experiment eight determinations were made over a period of 2 hours. The results of the six experiments are given in Table X; there is no trace of a consistent effect of the salt concentration.

TABLE X. Respiration of eel fins in 1/9 and in 5/6 s.w.

Exp. No.	Relative respiratory rates per g. per hour	
	In 1/9 s.w.	In 5/6 s.w.
F 1	100	93.5
F 2	100	102.0
F 3	100	121.0
F 4	100	99.0
F 5	100	85.5
F 6	100	113.0
Means	100	102.0

Eight determinations were made in each experiment.

s.w. = sea water.

The data presented in this paper have clearly the defect of some of the earlier experiments on the respiration of secretory tissue; they do not show, unequivocally, that the performance of work is causally associated with increased oxygen consumption. One can only point out that the tissue has a proven secretory capacity, and that the respiration does increase under conditions which are known to be associated with the initiation and maintenance of the secretion. Assuming, for the moment, that the secretion hypothesis is the correct one, we can make some more detailed observations on the experiments reported.

If the osmotic pressure of the internal medium really provides the stimulus for the secretion, then we must be able to show that the necessary change in the internal environment in the case of the blood-filled gills can occur early in the experiment in which the external medium is 5/6 sea water. We may use data for the permeability of the perfused gills when fresh water is the external medium [Keys, 1931 b]. These data indicate a water entry of about 5 c.c. per kg. eel per hour. In eels of the size used in the present experiments this means an entry of roughly 0.15 c.c. of water per hour per gill. The gill volume may be put at 0.5 c.c. (probably less) of which 20 p.c. is osmotically inert substance. It is clear that the normal permeability will effect in 1 hour a change of at least 35 p.c. in the internal concentration. Where the concentration gradient is greater, as in the case of gills suspended in 5/6 sea water, the effect of passive diffusion will be proportionally greater. The permeability of the extirpated gills, moreover, may be taken to be considerably greater than that of the gills *in situ*, in the same way that any tissue subjected to manipulation shows an increased permeability. It is obvious that sufficient exchange will take place in the early stages of the experiment to stimulate or stop the secretory activity as the case may be. The

results, then, are consistent with the opinion that the internal osmotic pressure is the stimulus for the secretion. This conclusion has been reached also from the recent experiments by Krogh and Schlieper [1932].

The respiration increment due to, or associated with, higher salt concentration is an excess of oxidation over the "resting" value in the lower salt concentration, and the excess energy yielded in this way may be calculated and compared with the medium energy cost of the gill secretion observed in the perfusion experiments. If it could be assumed that the salt concentration stimulus evoked the same degree of secretion activity in the extirpated gills as in the perfused gills, such a calculation would provide an answer to the question as to the efficiency of the secretory mechanism. Actually, of course, it is highly probable that the secretory activity in the extirpated gills is smaller, perhaps much smaller, than in the perfused gills, and the result of our calculation will be of interest only as a limiting value.

The mean value for the "secretion" increment in all experiments is 102.6 c.mm. O_2 per g. per hour. Assuming that carbohydrate is burnt, the combustion of this amount of oxygen would yield 0.51 cal. The secretion observed in the perfusion experiments involves a performance of thermodynamic work amounting to 0.1–0.3 cal. [Bateman and Keys, 1932], with the mean value not far from 0.15 cal. The first approximation to the maximum efficiency is therefore 29 p.c.

Organs capable of specialized activity frequently have a high resting metabolism. It is of interest, therefore, that the respiration of eel gill tissue in a dilute medium—about 210 c.mm. O_2 per g. per hour at 15° C.—is proportionally considerably greater than that of the mammalian lung. Lovatt Evans [1912] gives 500¹ c.mm. O_2 per g. per hour at 36° C. for the respiration of the lung tissue in the heart-lung preparation, and this works out to be about 1.5 times the rate for the body as a whole. The respiration rate of the "resting" gills is about seven times that of the resting intact eel, as measured by Krogh [1904], while the respiratory rate of the gill in the concentrated medium is about ten times that for the entire animal. Moreover, if allowance is made for the temperature difference by the application of the usual temperature coefficient— Q_{10} about 2.0—the respiratory rates of "resting" and "active" gills become respectively 100 p.c. and 190 p.c. greater than the rate of the mammalian lung tissue. For purposes of comparison, we have

¹ The value obtained from Evans's paper by Bayliss [1924, p. 612] appears to be incorrect.

assembled data on the respiratory rates of various mammalian and eel tissues in Table XI.

TABLE XI. Respiration of various tissues.
Respiration rates in c.mm. O₂ per g. per hour of wet tissue.

Tissue	Mean respiratory rate	Observer
Mammalian kidney	1600-3600	Neumann, 1912
Mammalian kidney	Approx. 1680	Barcroft and Brodie, 1905
Mammalian suprarenal gland	2750	Neumann, 1912
Mammalian submaxillary gland	1920	Verzár, 1912
Mammalian submaxillary gland	1620	Barcroft and Piper, 1912
Mammalian lung	500	Evans, 1912
Mouse and rat diaphragm	1000-2000	Meyerhof, 1930
Frog, small muscles at 14-8° C.	59	Fenn, 1927
Eel, pectoral fin at 15° C.	78	Original
dorsal fin (strips) at 15-1° C.	61	"
central fin (strips) at 15-1° C.	67	"
mid gut (strips) at 15-3° C.	63	"
blood-filled gills in 1/9 s.w. at 14-8° C.	167	"
blood-filled gills in 5/6 s.w. at 14-8° C.	273	"
gills after perfusion, in 1/9 s.w. at 15-0° C.	210	"
same calculated for 37° C.*	1000	"
gills after perfusion, in 5/6 s.w. at 15-0° C.	302	"
same calculated for 37° C.*	1430	"

* Assuming Q_{10} is 2.0.

SUMMARY.

1. The respiration of excised eel gills, filled with Ringer's solution and blood, has been measured in dilute and concentrated external environments.

2. The percentage of gill filament tissue in the gills has been determined and the respiration of the bars minus the filaments measured.

3. The mean oxygen uptake of the gills filled with Ringer's solution, corrected for the respiration of the relatively inert cartilaginous bars, was 210 c.mm. O₂ per g. per hour with 1/9 sea water as the external medium and 300 c.mm. with 5/6 sea water. In all experiments the respiration was consistently greater in the more concentrated medium.

4. With the blood-filled gills an even greater difference was found between the respiration in 1/9 and 5/6 sea water, the respiration in the latter averaging 80 p.c. greater than in the former.

5. Possible causes of this concentration effect are discussed and reasons are given for the belief that it is related to secretory activity in the gill. The possible efficiency of this activity is discussed.

6. The fins of the eel do not show any effect of osmotic concentration of the environment on the respiratory rate.

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THE MUCUS FACTOR IN THE AUTOMATIC
REGULATION OF THE ACIDITY OF
THE GASTRIC CONTENTS.

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THE automatic regulation of the gastric acidity is the result of the interaction of several factors. These are (1) intragastric, which are brought into play by the stomach itself; and (2) extragastric, which originate from without, that is to say from the duodenum and oesophagus. Study of a mass of clinical cases demonstrates quite clearly that each of these factors is of varying importance in different pathological conditions. It is, therefore, essential for the elucidation of such conditions that, in the first instance, the part played by each of these factors in the normal individual should be precisely defined. This research is concerned with the intragastric factors alone and more particularly with gastric mucus. This factor has hitherto been largely disregarded in favour of a hypothetical diluting solution, which has recently been regarded by some as a secretion [Maclea and Griffiths, 1928], in fact gastric juice in which the HCl has been replaced by NaCl. As a preliminary study of the intragastric factors, we, therefore, investigated the acidity of the gastric juice during a secretory wave and were able to prove that the original view of Pavlov was correct [Bolton and Goodhart, 1931; Pavlov, 1910]. We found that the HCl content of the gastric juice as secreted by the cell varies a little with the rate of secretion, but that the main cause of the moderate variations in acidity which occur during secretion is neutralization and dilution by mucus. Neither by any automatic conversion of HCl into NaCl by the secreting cell, nor by admixture with mucus is the unaided stomach able to reduce materially the acidity of the gastric juice during its secretion. We showed, however, that when secretion has stopped the reaction of the residual fluid in the stomach gradually becomes alkaline owing to dilution and neutralization with mucus. Since mucus is thus, in some degree, a factor of importance and

the precise part played by it is unknown, the next step in our study is clearly to determine the degree to which mucus is capable of neutralizing solutions of HCl of different amounts and strengths.

METHOD.

Cats were used, and in all the experiments the stomach was isolated from the rest of the alimentary canal by ligature of the cardia and pylorus. For all details of preparation of the cat the reader is referred to the above-mentioned communication [Bolton and Goodhart, 1931]. It is merely necessary to mention here that the HCl solution was introduced into the isolated stomach through the side tube of a wide cannula, the mouth of which was closed with a cork. The residual fluid, which would not flow out of the stomach after emptying it, was removed by a small glass syringe and piece of tubing introduced into the mouth of the cannula, through which easy access to all parts of the stomach was assured. The fluid was measured on introduction and after extraction. It is not possible to remove the whole of the fluid with certainty, owing to small amounts being held up by the folds of mucous membrane. Sometimes the measurements are the same, but usually a fraction of a c.c. less than the volume introduced is extracted in spite of the addition of a little mucus. If any considerable amount of fluid is added by the stomach, however, it can be recognized quite easily.

A fresh cat was used for each experiment, so that the compensatory changes incident to a permanent operation and liable to lead to erroneous results were avoided, and also each animal was in a thoroughly healthy condition. A large number of animals, to allow for normal variations, was used, and the stomach of each was examined after the experiment.

I. NEUTRALIZATION EFFECTS AS REGARDS VOLUME AND CONCENTRATION OF HCl SOLUTION.

In studying the effects of mucus, one must be quite certain that the gastric glands are at rest and remain so during the experiment. Although the cat has been isolated and has received no food for 24 hours, the mucous membrane of the stomach is acid in reaction in a considerable number of animals, and there is no certainty of its becoming alkaline within a reasonable time. Furthermore, we have found, as will appear later, that the secretion of gastric juice may continue or be excited by the acid solution introduced. In order to produce a pure mucous effect we, therefore, administered atropine gr. 1/10 subcutaneously after the

animal had been prepared. The secretion of gastric juice is thus promptly stopped, that of mucus continuing, and the mucous membrane gradually becomes alkaline first to dimethylamidoazobenzol and later to litmus. On the average it took about 25 min. before alkalinity to litmus was developed; variations about this average occurred due to different rates of mucus secretion, which varies from cat to cat. It is obvious that the longer the acid solution remains in the stomach the greater will be the reduction of its acidity; and also that the reduction to alkalinity of even small quantities would occupy a time outside the limits of our experiments. For comparison of the effects upon the different solutions the standard time of a quarter of an hour was selected, during which the fluid was allowed to remain in the stomach. It was then removed for analysis and replaced by a similar amount of fresh solution of the original strength; a curve of reduction of acidity was thus produced. The concentration of the solutions was approximately that of gastric juice, and also a descending series below this level.

GROUP 1. ATROPINE EXPERIMENTS. (TWENTY-ONE EXPERIMENTS.)

(1) *Acidity of HCl solution introduced of the same concentration as gastric juice.*

Acidity 0.140–0.144 N. Total Cl 0.14 N (0.5 p.c.) (Fig. 1).

When the amount introduced is 10 c.c. a slight fall in acidity occurs of about 0.01 *N*; the total Cl also falls by about 0.004 *N* (0.014 p.c.). Larger amounts show less effect still. In view of this fact four experiments were performed using amounts of 10, 5 and 2 c.c. introduced every quarter of an hour.

Amount 10 c.c. The quarter-hourly curve remains horizontal as regards both acidity and total Cl. The same amounts as those introduced were extracted or a fraction of a c.c. less.

Amount 5 c.c. The same result was obtained on two occasions. The acid curve was reduced by 0.05 *N* at the beginning and by 0.03 *N* at the end, and therefore showed a slight gradual rise. The total Cl curve was horizontal and only reduced by about 0.01 *N* (0.036 p.c.); the first two or three specimens being slightly more affected than the others. The same amount or a fraction of a c.c. more than introduced was withdrawn on each occasion.

Amount 2 c.c. The acid curve was reduced by 0.06 *N* at the beginning and 0.05 *N* at the end, and the total Cl curve by about 0.014 *N* (0.043 p.c.).

The reduction in acidity is, therefore, only a marked feature when

amounts below 10 c.c. are introduced and is a little less after repeated introduction. This latter event is due to continual washing of the mucous membrane with acid and is without doubt the same phenomenon as is observed in the acidity of the secretory curve, which is lower at the beginning than the end of secretion. With weak solutions of acid this effect is not seen. On the other hand the total Cl curve falls but little, and practically to the same degree in each case. This is owing to the high Cl content of mucus, which is only slightly less than that of gastric juice, and which, therefore, acts only feebly as a diluting fluid.

(2) *Acidity of HCl solution introduced of a concentration below that of the gastric juice.*

Acidity 0.1–0.11 N. Total Cl 0.1–0.11 N (0.36–0.39 p.c.) (Fig. 2).

Amount introduced 10 c.c. The acidity curve was reduced by about 0.010 N, but the total Cl curve was unaffected.

Amount 5 c.c. Both curves were horizontal, the acidity being reduced by 0.03 N and the total Cl raised by from 0.004 to 0.008 N (0.014–0.028 p.c.).

Amount 2 c.c. The reduction of acidity was about 0.06 N and the rise of total Cl 0.004–0.008 N (0.014–0.028 p.c.).

The acidity showed a greater reduction with the smaller amounts than the larger, and to about the same degree as when the concentration of the HCl was 0.140 N. On the other hand the total Cl was raised a little in the case of the smaller amounts, since the diluent, mucus, has a greater concentration of total Cl than the fluid introduced.

Acidity 0.068–0.072 N. Total Cl 0.06–0.07 N (0.24–0.25 p.c.) (Fig. 3).

Amount introduced 10 c.c. The acid curve was again reduced slightly by about 0.004–0.01 N and the total Cl raised by about 0.004–0.008 N (0.014–0.028 p.c.).

Amount 5 c.c. The reduction of acidity was by about 0.02 N and the rise in total Cl by 0.02–0.03 N (0.07–0.1 p.c.).

Amount 2 c.c. The result was almost the same as in the preceding experiment. Reduction of acidity by 0.03 N and rise in the total Cl by 0.02 N (0.07 p.c.).

It is to be noted that the reduction of acidity is less than in the case of the same amounts of the stronger solutions, but the rise of total Cl is more, owing to the greater difference between the concentration of Cl in the introduced fluid and in mucus.

Acidity 0.036–0.044 *N*. Total Cl 0.036–0.044 *N* (0.129–0.15 p.c.) (Fig. 4).

Amount introduced 10 c.c. The acidity was reduced by 0.015 *N* in one case and by 0.004 *N* only in the other. On the other hand the total Cl percentage went up by 0.036 in the former and 0.1 in the latter.

Amount 5 c.c. The fall in acidity was 0.02 *N* in one case, but only 0.004–0.008 *N* in the other. The total Cl percentage rose by 0.1 in the former and 0.07 in the other.

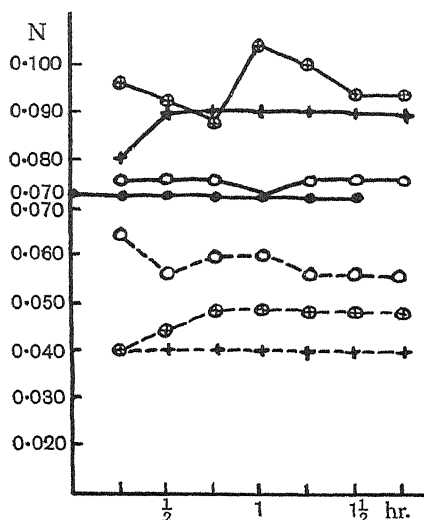


Fig. 3. Cat under atropine. 0.072 *N* HCl introduced every $\frac{1}{4}$ hour. Symbols as in Fig. 1.

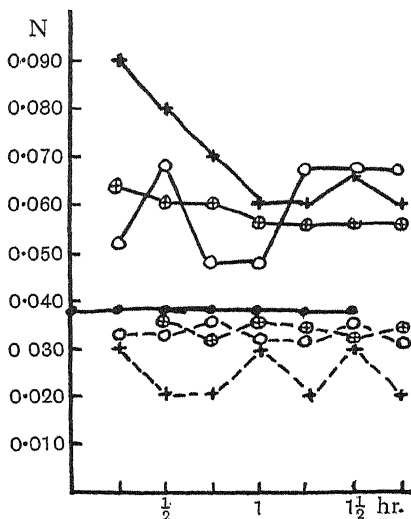


Fig. 4. Cat under atropine. 0.038 *N* HCl introduced every $\frac{1}{4}$ hour. Symbols as in Fig. 1.

Amount 2 c.c. The reduction in acidity was only 0.006–0.016 *N* and the rise in total Cl percentage by 0.19 at the beginning, and by 0.08 at the end.

Generally speaking, in the case of strong solutions up to the strength of gastric juice, both acidity and total Cl fall, the former more than the latter; and the smaller the volume of solution the greater the fall in acidity, but the total Cl fall remains about the same in all cases. In the case of weak solutions the acidity falls, but less than with strong solutions, and the total Cl rises. The weaker the solution the less the fall in acidity and the greater the rise in total Cl. The difference between the effects upon acidity in the case of large and small amounts of weak solutions

is less marked than in the case of the strong solutions: but the total Cl is always higher with small amounts of weak solutions. However small the volume of stomach contents the reaction is invariably acid, and after complete emptying the mucous membrane becomes alkaline in about half an hour. These effects are all referable to two facts: (1) The total Cl content of mucus which is the diluent is very little less in most cases than that of gastric juice, so that below a certain level of total Cl content of introduced solution, say 0.36 p.c., the rise in total Cl is greater the weaker the solution and the smaller the amount present. (2) The neutralizing power of mucus depends upon the relative amount present. The smaller the amount of solution the greater the relative amount of mucus. The stronger the solution the greater the flow of mucus, and the greater its relative amount.

(3) *Reproduction of secretory curve.*

This demonstration that the gastric mucus lowers the acidity of definite amounts of definite strengths of HCl solution to approximately definite levels, and that so long as the acid solution is renewed the level remains constant, implies that a gradually rising and falling curve of acidity can be produced by appropriately varying the amounts of the acid solution. In order to test this implication a series of four experiments was undertaken, atropine gr. 1/10 being administered to produce alkalinity of the mucous membrane.

The concentration of the HCl solution was 0.14–0.144 *N*, the strength of gastric juice. The amounts introduced each quarter of an hour were 2, 5, 4, 3, 2 c.c. in two cases, 2, 6, 4, 3, 2 c.c., and 3, 6, 5, 4, 3 c.c. in the other two cases (Fig. 5). The acidity fell from its initial height in two cases to 0.08 *N* and in the others to 0.06 and 0.07 *N* respectively during the first quarter-hour period. At the end of the experiment the acidity of the final amounts extracted in three cases stood from 0.01 to 0.024 *N* higher than at the beginning: in the final case it stood at 0.12 *N*, 0.05 *N* higher than at the beginning and remained at this level after three further introductions of the same amount (2 c.c.). A rising and a falling acidity curve was thus produced in three cases and a climbing curve in the other, the final acidity being always higher than at the beginning of the curve. The total Cl curve was about 0.01 *N* (0.036 p.c.) or less below the level of that introduced and was a horizontal line in three cases: in the other case it fell finally to 0.024 *N* (0.084 p.c.) below the initial level. This fall was accounted for by the low Cl content of the mucus of this animal: the mucous membrane after emptying the stomach became

alkaline in half an hour, and the mucus extracted contained only 0.36 p.c. inorganic chloride, rather lower than usual.

These four experiments reproduced precisely the same type of curve as is obtained during the secretion of gastric juice. The effect of the mucus was, however, more marked, so that the acidity curve ran at a lower level than is usual with the gastric secretory curve. The explanation is obviously that the gastric juice, secreted as a continuous process, has to pass through the overlying mucus which it saturates before appearing free in the stomach, so that, as Pavlov puts it, the mucous membrane becomes well washed. This is a more thorough process than the intermittent introduction of acid solution into the stomach lined with mucus, so that the neutralization effect is less marked in the case of the secreted acid.

GROUP 2. CONTROL EXPERIMENTS WITHOUT ATROPINE.
(SIX EXPERIMENTS.)

In all the above experiments under atropine the only secretion by the stomach was mucus to which the neutralization effect was due; and, furthermore, after the stomach had become emptied, this was proved to be the case by collecting the subsequent secretion. It is still possible that the stomach might secrete a neutral diluting fluid unless stopped by the atropine. A series of similar experiments was, therefore, performed without using atropine, the HCl solution introduced being of the same strength as gastric juice, about 0.14 *N*. The stomach was very gently mopped free of residual fluid with absorbent cotton wool before introducing the solution. If the mucous membrane was acid in reaction we found that it might remain so for a couple of hours, so we made no attempt to wait till it became alkaline, but introduced the solution irrespective of the initial reaction of the mucous membrane. We shall return to this point later when describing another series of experiments. There were three experiments in which the same amounts—20, 10 or 5 c.c.—were repeatedly introduced and three in which increasing and diminishing amounts were used—2, 5, 4, 3, 2 c.c., 3, 6, 5, 4, 3 c.c. (Fig. 5), and 2, 4, 3, 2 c.c. The amounts extracted on each occasion were similar to those in the experiments under atropine.

Amount 20 c.c. There was a very slight reduction of acidity of 0.004 *N*, an insignificant amount (Fig. 6).

Amount 10 c.c. There was a great deal of mucus in this case and the reduction of acidity at the beginning was 0.028 *N* and at the end of the curve 0.008 *N* (Fig. 6).

Amount 5 c.c. The acidity of the first specimen was reduced by 0.036 *N* and at the last by 0.02 *N* (Fig. 6).

Each of the second three experiments reproduced the type of curve which we have called the secretory type, the final acidity being less reduced for the same amount of solution than the initial was. For example the cases showed:

Amounts c.c.	Acidity of first specimen <i>N</i>	Final acidity <i>N</i>
2	0.1	0.11
3	0.09	0.11
2	0.05	0.08

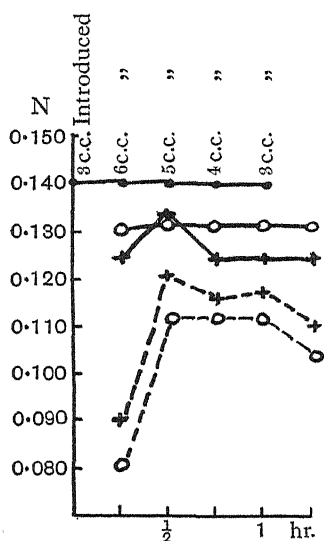


Fig. 5.

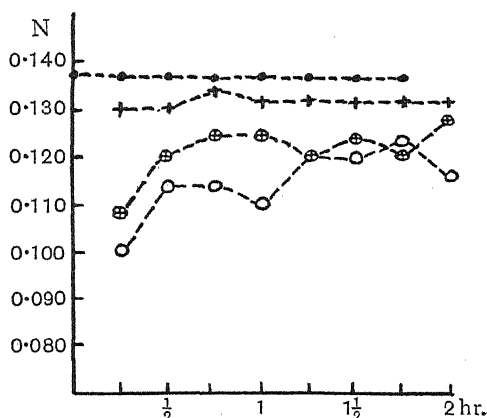


Fig. 6.

Fig. 5. Reproduction of secretory curve by introduction of increasing and diminishing amounts of 0.14 *N* HCl.

Cat under atropine, gastric contents: total Cl ○—○—○
 total acid ○---○---○
 Cat without atropine, gastric contents: total Cl +—+—+
 total acid +---+---+
 Total Cl and total acid introduced ●—●—●

Fig. 6. Control cats without atropine. 0.137 *N* HCl introduced every $\frac{1}{4}$ hour.

Total acid introduced ●---●---●
 Gastric contents: 20 c.c. introduced, total acid +---+---+
 10 c.c. " " ⊕---⊕---⊕
 5 c.c. " " ○---○---○

These results are the same as those in the atropinized animal and were very uniform considering the fact that slight acid secretion was occurring at the time of the experiment in each case. It is reasonable to conclude that the neutralization effect in these cases was due to mucus and that no neutral diluting secretion was concerned.

II. NEUTRALIZATION EFFECTS IN RELATION TO THE EMPTYING OF THE STOMACH.

We have hitherto considered the effects of mucus upon HCl solution of different amounts and strengths which were constantly renewed. The results of these observations are directly applicable to the pathological condition of hypersecretion of gastric juice into the empty stomach at different rates. If the rate of secretion is maintained the curve of acidity is a horizontal line at different levels according to the amount of juice put out in unit time. In the condition of digestion in the normal stomach, however, the flow of gastric juice comes to an end more or less rapidly and the contents of the stomach are diminishing as the food leaves it. The next problem which confronts us, therefore, is to ascertain the type of curve which is produced by mucus as the stomach empties when the flow of juice has stopped; it is obvious that as the flow of juice is diminishing the curve will fall similarly, but at a much slower rate, for, so long as any secretion continues and acid is being added, mucus can only lower the acid curve to a certain level depending on the rate of secretion and the amount of gastric contents. Stated in experimental terms our task is to discover to what extent mucus is able to dilute and neutralize a solution of HCl which is gradually diminishing in volume, and what types of curve are produced with different strengths of acid. In this series of experiments a measured amount of HCl solution was introduced into the stomach, and each quarter of an hour the whole of the solution was removed and measured. A certain volume was retained for analysis and the remainder returned into the stomach. The concentration of acid used was that of the gastric juice, also weaker and stronger solutions. Two groups of experiments were performed: (1) under atropine, to obtain a pure mucus effect; (2) a control series, without atropine.

GROUP 1. UNDER ATROPINE gr. 1/10. (EIGHT EXPERIMENTS.)

Concentration of HCl = 0.140–0.144 *N*. *Total Cl* 0.14–0.144 *N* (0.50–0.51 *p.c.*) (Fig. 7).

When the mucous membrane had become alkaline 50 c.c. of the solution were introduced, and a quarter of an hour later the stomach

was emptied; 40 c.c. of the fluid thus removed were immediately returned to the stomach, and each quarter hour subsequently the process was

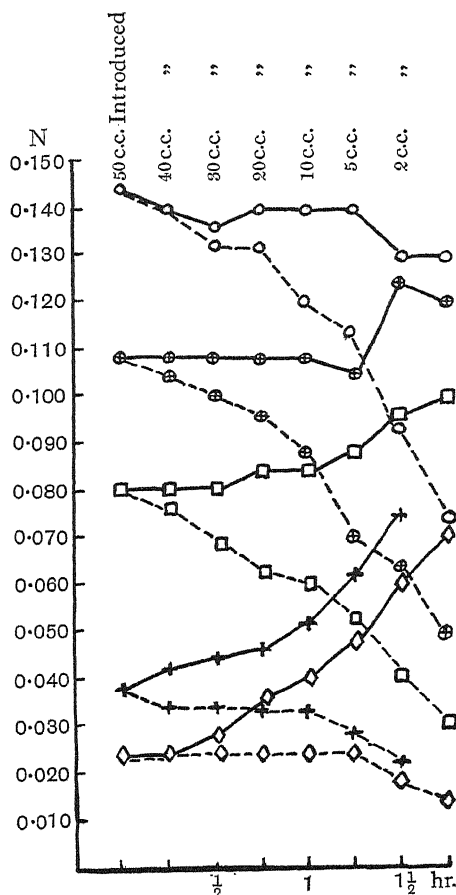


Fig. 7. Five cats under atropine. 50 c.c. HCl, 0.144 N, 0.108, 0.080, 0.040, 0.024 N introduced; stomach emptied at $\frac{1}{4}$ hour and 40 c.c. of gastric contents put back at once, and so on. Gastric contents: total chloride, continuous line; total acid, interrupted line.

repeated, 30, 20, 10, 5 and 2 c.c. being returned at each successive period. The total Cl curve was constant till the amount diminished to 10 or 5 c.c., when it fell by about 0.036 p.c. The acid curve began to fall slightly at the 20 or 10 c.c. amounts: at the amount 5 c.c. it had reached 0.097-

0.1 *N* and at 2 c.c. 0.075 *N*. These levels correspond quite well with those of the earlier experiments.

Concentration = 0.108 *N*. *Total Cl* 0.108 *N* (0.38 p.c.) (Fig. 7).

The same amounts as before were successively introduced in this and all the following experiments. The total Cl curve remained at the original level till the amount 5 c.c. was reached, when it rose to 0.123 *N* (0.44 p.c.) and finally stood at 0.117 *N* (0.42 p.c.). The acidity curve fell slightly and steadily to 0.088 *N* till the amount 20 c.c. was reached; it then fell rapidly, finally standing at 0.05 *N*.

Concentration = 0.08 *N*. *Total Cl* 0.08 *N* (0.28 p.c.) (Fig. 7).

The total Cl and acidity curves began to diverge as in the last case, the former to rise and the latter to fall, when the amount 30 c.c. was reached; and at 2 c.c. the Cl curve had risen to 0.1 *N* (0.36 p.c.) and the acidity curve fallen to 0.03 *N*.

Concentration = 0.038 *N*. *Total Cl* 0.038 *N* (0.136 p.c.) (Fig. 7).

The total Cl and acidity curves began to diverge slightly at once, and more rapidly at the amount 20 c.c.; at 5 c.c. the total Cl curve had risen to 0.073 *N* (0.26 p.c.), and the acidity curve fallen to 0.022 *N*.

Concentration = 0.024 *N*. *Total Cl* 0.024 *N* (0.084 p.c.) (Fig. 7).

The two curves began to diverge at the amount 30 c.c., and at 2 c.c. the Cl curve had risen to 0.07 *N* (0.25 p.c.); the acidity curve remained at its original level till the amount 5 c.c., and at 2 c.c. it fell to 0.014 *N*. Here again in this series the fact is illustrated that lower concentrations of HCl are reduced in acidity less than higher concentrations. In this experiment after emptying the stomach, the mucous membrane became alkaline in 20 min.

Concentration of HCl above that of gastric juice. Acidity 0.216 and 0.276 *N*. *Total Cl* 0.216 and 0.276 *N* (0.77 and 0.99 p.c.).

In each case the amounts introduced were 50, 30, 10, and 5 c.c.

The results of these two experiments differ from the foregoing in the following particulars:

Concentration 0.276 *N*:

(1) From 1 to 2 c.c. were extracted in excess of that introduced on each occasion.

(2) The diluting fluid was an inflammatory effusion containing albumin and blood.

(3) From the beginning, when the amount was 50 c.c., there was a sharp fall of both total Cl and acidity, the latter outpacing the former. At the end of the experiment the total Cl stood at 0.16 *N* (0.561 p.c.), having fallen from its original height of 0.276 *N* (0.993 p.c.); the acidity had meanwhile fallen to 0.1 *N*.

Concentration 0.216 *N*. This case showed less marked features than the former:

- (1) An excess of fluid was removed in the last two specimens only.
- (2) The diluting fluid was an inflammatory effusion containing blood.
- (3) There was a steady fall of both curves from the beginning, but only as steep as the former case after reaching the amount 10 c.c.; the final total Cl level was 0.172 *N* (0.619 p.c.), having fallen from 0.216 *N* (0.77 p.c.); and the final acidity was 0.116 *N*. So that the acidity fell to practically the same level in both cases and the final total Cl percentage only differed by 0.05 p.c.

The following deductions may be drawn from a consideration of this series of cases:

(1) When the total Cl content of the solution is greater than that of mucus the total Cl curve falls, the rapidity of fall depending upon the strength of the solution.

(2) When the total Cl content of the solution is less than that of mucus the curve rises.

(3) In each case when alkalinity is reached after emptying the stomach, the total Cl level stands at that of mucus, or of the inflammatory effusion in the case of solutions much stronger than gastric juice.

(4) This fall or rise to a common level of Cl is not due to absorption or output of Cl in order to regulate the Cl content of the solution in the stomach to a definite constant level. It is due to admixture with mucus, and the final Cl percentage is that of mucus, or in the case of very strong solutions to admixture with inflammatory effusion, the Cl content of which is about the same as mucus.

(5) The rapid fall with very strong solutions, and the less rapid fall with solutions of the strength of gastric juice and of strengths above it to a certain height, are due to the quantity of diluting and neutralizing fluid. This quantity depends on the concentration of HCl in the solution and reaches its maximum in the cases showing inflammatory effusion.

(6) A whole series of diminishing degrees of both rapidity of fall, and final level reached, is seen in the acidity curves of the different strengths of acid:

(a) In the case of weak solutions up to the strength of gastric juice, a fall of acidity only occurs to any degree, when small amounts of solution are introduced. Above the strength of gastric juice the fall affects larger amounts of fluid, till, when the stage of inflammatory effusion is reached, a rapid fall occurs at the 50 c.c. amount.

(b) The rate of fall gradually diminishes and the curve becomes less steep from the higher to the lower strengths.

(c) The degree of fall, and the final level reached, is less and less marked as the strength of the HCl diminishes.

There is thus a whole series of irritation effects produced according to the strength of the HCl; for it is only reasonable to conclude, that the increasing fall of acidity with increasing strengths of HCl, when mucus secretion alone is excited, is due to increasing irritation, since the series passes up gradually into the cases of inflammatory effusion obviously due to local irritation of the HCl.

GROUP 2. CONTROL CASES WITHOUT ATROPINE. (THIRTY EXPERIMENTS.)

In this second group of experiments the solutions were introduced into the isolated normal stomach after removing any fluid which might have been present. Atropine was not administered, so the results observed were comparable to the neutralization effects which may reasonably be expected to occur during the emptying of the normal stomach, as the result of intragastric factors alone. This series of experiments is thus of great importance. There were three series as in the atropine cases. The amounts introduced were 50, 40, 30, 20, 10, 5 and 2 c.c.

(A) Concentration of acid similar to that of normal gastric juice, 0.135–0.152 *N*.

(B) Concentration of acid below this level, 0.02–0.12 *N*.

(C) Concentration of acid above this level, 0.188–0.292 *N*.

(A) *Concentration of acid similar to that of normal gastric juice,*
0.135–0.152 *N*. Total Cl 0.135–0.150 *N* (0.48–0.54 p.c.)

(Figs. 8 and 9).

Precisely the same results were obtained as in the atropine cases. The total Cl curve remained almost unaffected till the amount 10 c.c. was reached, when a fall began, the height standing at 0.125 *N* (0.45 p.c.) when the amount 2 c.c. was reached, a fall of about 0.023 *N* (0.08 p.c.). The acid curve was very little affected till the 10 c.c. amount, when it began to fall rapidly, reaching the level of about 0.09 *N* at the 5 c.c. amount, and 0.07 *N* at the 2 c.c. amount. In two cases the emptying

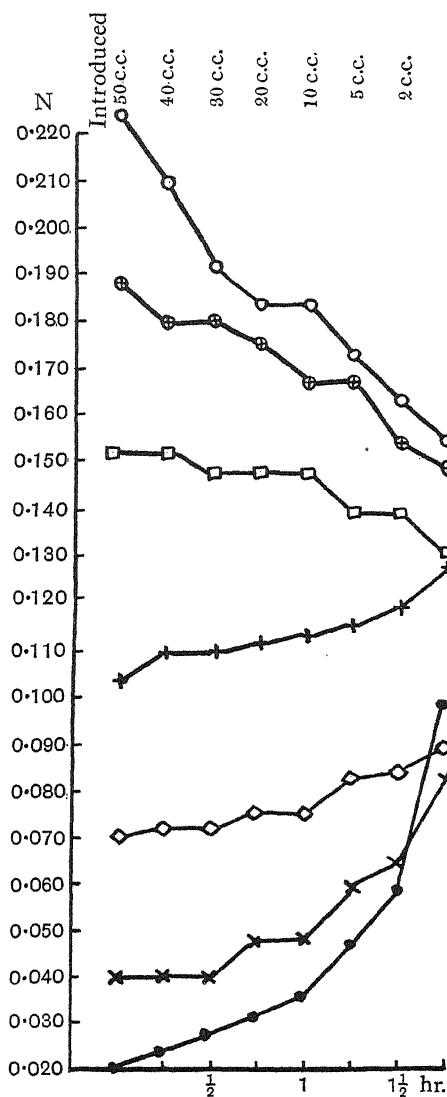


Fig. 8. Seven cats. Control experiments of Fig. 7 without atropine.
Gastric contents, curves of total Cl.

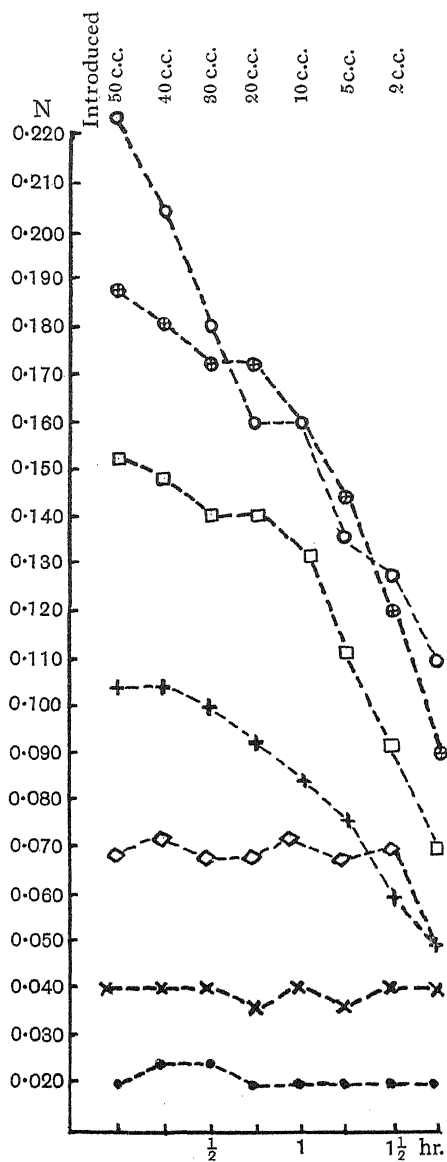


Fig. 9. Same as Fig. 8. Curves of total acid.

time was lengthened by 1 hour, by withholding smaller amounts than usual each quarter hour. In one case the 2 c.c. amount was reached at the end of $2\frac{3}{4}$ hours, and in the other the 20 c.c. amount was reached in $2\frac{1}{4}$ hours. In each the total Cl curve was very little affected, falling by 0.06 in one and 0.08 p.c. in the other. In each case there was a steady uniform fall of acidity, to 0.04 *N* in the former case and to 0.09 *N* in the latter. In each case there was a greater fall of acidity than occurred with similar amounts when the emptying was more rapid, but the fall was more gradual. This was clearly due to the longer time which was allowed for the action of the mucus. We may conclude that the quicker the emptying the more rapid and less complete the fall; the slower the emptying the less rapid but more complete the fall. The rate of emptying of the stomach is, therefore, an intragastric factor which influences the acidity of the contents and in the direction just mentioned. Furthermore, there appears to be no special level to which the acidity is regulated by the mechanisms of emptying time and mucus secretion, the final level is fortuitous, and depends upon relative amounts of acid solution and mucus.

(B) *Concentration of acid below 0.02–0.12 N.*

Total Cl 0.02–0.12 N (0.07–0.43 p.c.).

Concentration of acid = 0.12 N. Total Cl 0.12 N (0.43 p.c.).

This experiment gave the same result as the atropine experiments, in that the total Cl curve at the 2 c.c. level rose to 0.13 *N* (0.46 p.c.) and the acidity curve fell to 0.05 *N*. After emptying the stomach, 0.5 c.c. mucus was collected in the next quarter-hour period and showed an acidity of 0.02 *N*, so that neutralization by the mucus was not yet complete. This, as we have already mentioned, takes on the average half an hour.

Concentration = 0.096–0.104 N. Total Cl 0.09–0.104 N (0.32–0.374 p.c.) (Figs. 8 and 9).

Typically the total Cl curve rose to 0.11–0.117 *N* (0.396–0.42 p.c.) at the 5 c.c. amount and to 0.125 *N* (0.45 p.c.) at the 2 c.c. amount. The acidity curve fell to 0.06–0.084 *N* at 5 c.c., and to 0.05 *N* at 2 c.c. This is a quite similar result to the corresponding case under atropine.

Concentration = 0.068–0.084 N. Total Cl 0.068–0.084 N (0.24–0.29 p.c.) (Figs. 8 and 9).

These curves showed a less marked effect than the corresponding case under atropine and did not diverge until the amount 10 c.c. was

reached. The total Cl rose to 0.084–0.1 *N* (0.29–0.36 p.c.) at the 5 c.c. amount. The acidity at this time in one case did not fall at all; in another it rose to 0.076 *N* from a level of 0.068 *N*; and in the others fell slightly to about 0.06 *N*.

Concentration = 0.02–0.04 *N*. *Total Cl* 0.02–0.04 *N* (0.07–0.14 p.c.) (Figs. 8 and 9).

These curves similarly differed from the atropine cases in showing no fall in acidity; for example, it showed no fall at all in three, and in one it rose slightly. The total Cl rose in all by from 0.02 to 0.04 *N* (0.07–0.14 p.c.) at the 5 c.c. amount. The stomach was emptied in one case, and after $\frac{3}{4}$ hour 1 c.c. of mucus was extracted showing an acidity of 0.07 *N* and total Cl of 0.14 *N* (0.5 p.c.).

These irregularities in acidity in the last two series can only be due to one thing, namely the secretion of acid gastric juice.

We now bring together the cases showing evidence of this secretion.

(1) The acid curve rises instead of falling. Two experiments:

(a) The acidity of the 50 c.c. amount introduced was 0.068 *N* and the total Cl 0.068 *N* (0.23 p.c.). When the 5 c.c. amount was reached the acidity had risen to 0.076 *N* and the total Cl to 0.08 *N* (0.288 p.c.).

(b) The acidity of the 50 c.c. of introduced fluid was 0.035 *N* and the total Cl 0.035 *N* (0.12 p.c.). When the 5 c.c. amount was reached the acidity stood at 0.045 *N* and the total Cl at 0.055 *N* (0.198 p.c.).

(2) In one case the secreted fluid which was collected $\frac{3}{4}$ hour after emptying the stomach had a higher acidity than the introduced fluid. The acidity of the original fluid introduced was 0.04 *N* and total Cl 0.04 *N* (0.14 p.c.). When the 2 c.c. amount was reached the acidity still remained at 0.04 *N*, and the total Cl had risen to 0.083 *N* (0.29 p.c.). The mucus, which was collected after emptying the stomach, had an acidity of 0.07 *N* and a total Cl of 0.14 *N* (0.5 p.c.).

(3) In three cases the acidity remained at the same level throughout, that is to say 0.04, 0.038, and 0.02 *N*. No fall in acidity occurred in any of the series of cases of low acidity, and although we cannot prove that this is due to secreted acid it is most likely to be the cause, for in no case did such an event occur in the atropine cases. When solutions of acidity stronger than that of the last series of cases is employed, it is not possible to say whether or not there is any secretion of gastric juice. It is only when the acidity is at this low level that the small amounts of gastric juice secreted could add to the acidity appreciably, otherwise it could do no more than retard the fall of the acid curve: or when the mucoid

fluid extracted after the stomach is emptied has an acidity greater than the original acidity can it be said that there would be proof that acid was being secreted.

It has already been mentioned that there is no evidence of any mechanism regulating the acidity of the gastric contents to any special level. It might be argued that the rise of acidity when weak solutions are introduced, and the fall of acidity with strong solutions, means that the stomach is trying to adjust the acidity of its contents to some intermediate level. This cannot be so, for most of the weaker solutions show no rise, the curve being a horizontal line; and when a rise occurs it is to no definite level. Also the fall of acidity in the case of the stronger solutions is finally to alkalinity, and does not stop at any definite level of acidity. In all cases when the stomach is emptied the secretion obtained is usually alkaline mucus, but may be mucus with some secreted gastric juice. Similar remarks apply to the total Cl curve which is not adjusted to any definite level, although it falls slightly in the case of strong solutions and rises with weak solutions. The final level when alkalinity is reached is somewhat variable, depending on the concentration of Cl in the mucus.

(C) *Concentration of acid* 0.188–0.292 *N*.

Total Cl 0.188–0.292 *N* (0.66–1.04 *p.c.*).

Concentration of HCl (0.188 and 0.196 *N*). *Total Cl* 0.188 and 0.196 *N* (0.66 and 0.7 *p.c.*) (Figs. 8 and 9).

Both these cases showed a fall in the acidity and total Cl curves from the beginning, but the fall in total Cl was quite gradual from start to finish, the final reading being, at the amount 5 c.c., 0.15 and 0.153 *N* (0.54 and 0.55 *p.c.*) in the two cases, so that they fell by 0.034 and 0.04 *N* (0.12 and 0.14 *p.c.*) respectively. The acid curve fell more rapidly after the amount 10 c.c., and at the 5 c.c. amount stood at 0.120 and 0.125 *N* in the two cases, so that they fell by 0.068 and 0.071 *N* respectively. This result is so nearly like that of Group 1 that it must be due to the same cause, and a comparison of the two curves leaves no doubt in the mind that the difference in the two cases is due to greater mucus secretion in the case of the stronger solution, owing to greater irritation. We could not collect enough mucus afterwards to prove that it did not contain albumin, but it will be seen later than albumin does not usually occur with these strengths of HCl.

Concentration = 0.224, 0.276, 0.292 *N*. Total Cl 0.224, 0.276, 0.292 *N* (0.8, 0.99, 1.047 *p.c.*) (Figs. 8 and 9).

In each case both the curves fell steeply from the beginning, more especially that of the highest strength. The two cases 0.276 and 0.292 *N* gave the same results, in that a greater amount was extracted than was introduced, more particularly at the amounts 40, 30 and 20 c.c. when it was from 3 to 6 c.c. in excess. This was proved to be due to admixture with an inflammatory exudate containing about the same amount of total chloride as mucus, that is 0.125 *N* (0.45 *p.c.*). At the 5 c.c. amount the total Cl had dropped to 0.142 and 0.148 *N* (0.51 and 0.53 *p.c.*): the total acidity at the same time stood at 0.05 and 0.06 *N* respectively. These results are practically the same as those of the atropine experiments. In the case 0.224 *N* no more was extracted than was introduced on each occasion, and we could not obtain sufficient mucus for examination, so are unable to say whether it contained albumin. When the 5 c.c. amount was reached the total Cl stood at 0.16 *N* (0.56 *p.c.*) and the acidity at 0.11 *N*; not such a great fall in acidity as with the stronger solutions.

Reviewing these three groups of experiments, it is plain that they show the same transition of results as occurs in the atropine cases. The fall in acidity is proportionate to the strength of the solution: the same fall in total Cl occurs in the strong solutions and the same rise in the weak solutions: the neutralizing and diluting fluid is the same in each case, having a quantity and quality proportionate to the strength of the acid; mucus with strengths some degree above that of gastric juice, and inflammatory effusion with higher strengths still. The only difference is that gastric juice is liable to be secreted, as shown in the case of the weaker strengths of acid; whether this is a continuation of the slow secretion going on in the fasting stomach of the cat, and unaffected by the HCl solutions introduced, or whether it is newly excited by the solution, perhaps by the water it contains, we are unable to say.

III. MUCUS.

This section deals with the direct evidence we have obtained that mucus alone is the diluting and neutralizing fluid of these HCl solutions, and that when a certain concentration of acid is reached an inflammatory effusion is added to the mucus. This inflammatory fluid is of no moment from the physiological point of view, but for our purpose it is of importance, because it is clearly due to irritation by the HCl; and since it

gradually becomes mixed with and succeeds the mucus secretion, and the effects of the one gradually pass into the other, it is only reasonable to suppose that the similar graduated series of effects due to mucus alone, and varying in accordance with the strength of the acid, are dependent upon the varying amounts of mucus secreted in response to the irritation of the HCl. Furthermore, these experiments show how comparatively easy it is to produce such an inflammatory effusion, which is of great importance in pathology.

When an irritant fluid is applied to the mucous membrane of the stomach a flow of mucus is excited, and continues for a time after the fluid is removed, so that it may be collected. This is the method we have employed for the collection of mucus in this series of experiments. It is, however, only on occasion that sufficient may be collected for analysis after removal of these solutions of HCl. The mucus is thick and sticky and considerable quantities adhere to the mucous membrane and are entangled in its folds, but it is only when it collects in a pool in the cavity of the stomach that it can be extracted. The stomach was emptied as far as possible and then any remaining fluid was gently removed with absorbent cotton wool. The mucous membrane was tested from time to time as to its reaction; it became alkaline first to dimethylamidoazobenzol, and about half an hour later to litmus. At this stage the mucus was allowed to collect until sufficient could be extracted. We succeeded in obtaining it from twenty cases, but in the rest, although the mucous membrane became alkaline, no definite quantity of fluid could be extracted. We have already referred to one case in which the mucous membrane did not become alkaline but secreted gastric juice, and have given further evidence of the secretion of this fluid, so need not refer to it again. Of the twenty experiments ten were performed under atropine and ten without atropine. There was no difference in the two series; in all cases mucus only was obtained or an inflammatory exudate containing albumin, and there was never any sign of the secretion of a neutral diluting fluid. There were eight cases of inflammatory exudate and twelve of mucus secretion. The amount of inflammatory exudate put out in from $\frac{1}{2}$ to 1 hour was 4-6 c.c. It contained albumin and often blood pigment, and the total Cl content was the same as that of mucus. Mucus is not secreted so abundantly; in five cases from 1 to 5 c.c. were collected in about an hour, and in seven no more than 0.5-0.6 c.c. The physical characters are the same as those of mucus obtained by other forms of irritation. The viscosity varies a little; sometimes the mucus runs well, at other times not. It is slightly opaque and contains a pro-

teose, and the alkalinity was 0.04 *N* (0.14 p.c.). The chloride content more particularly concerns us, and the analysis of the five above-mentioned cases is as follows:

Amount of mucus collected c.c.	Total Cl concentration	Concentration of HCl
1	0.1 <i>N</i> (0.36 p.c.)	0.04 <i>N</i>
1.5	0.12 <i>N</i> (0.43 p.c.)	0.044 <i>N</i>
1	0.14 <i>N</i> (0.50 p.c.)	0.07 <i>N</i>
1.5	0.12 <i>N</i> (0.43 p.c.)	0.104 <i>N</i>
1	0.131 <i>N</i> (0.47 p.c.)	0.148 <i>N</i>

The analysis of the seven cases in which smaller amounts were collected, and which are not, therefore, so reliable, is as follows:

Amount of mucus collected c.c.	Total Cl concentration	Concentration of HCl
0.6	0.12 <i>N</i> (0.43 p.c.)	0.02 <i>N</i>
0.7	0.14 <i>N</i> (0.50 p.c.)	0.038 <i>N</i>
0.3	0.128 <i>N</i> (0.46 p.c.)	0.140 <i>N</i>
0.4	0.11 <i>N</i> (0.39 p.c.)	0.144 <i>N</i>
0.5	0.1 <i>N</i> (0.36 p.c.)	0.144 <i>N</i>
0.5	0.12 <i>N</i> (0.43 p.c.)	0.145 <i>N</i>
0.6	0.14 <i>N</i> (0.50 p.c.)	0.180 <i>N</i>

The chloride content of gastric mucus excited by various strengths of HCl, therefore, varies from 0.36 to 0.5 p.c., and is independent of the strength of the HCl solution. This Cl content of mucus explains the rise or fall of total Cl in the introduced HCl solutions, which we have already described. We found the same percentage of total Cl in the mucus secreted in response to pilocarpine stimulation. The concentration of HCl giving rise to inflammatory exudation is about 0.19–0.2 *N* and all strengths above this level. In such cases hæmorrhages and erosions to various extents were seen in the mucous membrane of the stomach, or recognized microscopically.

IV. SUMMARY AND CONCLUSIONS.

1. The only means possessed by the normal stomach, whereby it is able to reduce the acidity of its contents, is by the secretion of mucus.
2. The action of mucus is influenced by two additional intragastric factors; the rate of emptying of the stomach and most important of all the cessation of secretion of gastric juice.
3. The more rapidly the stomach empties the more rapidly does the curve of acidity fall, notwithstanding that a shorter time is allowed for the action of the mucus than in the slowly emptying stomach. The

amount of gastric contents is thus of greater importance than the time allowed for the action of mucus in this neutralization process.

4. As regards amount to be neutralized, there is very little reduction of acidity till the amount is reduced below 10 c.c. in the cat.

5. As regards time for the action of mucus, a greater final reduction of acidity occurs for any definite amount of fluid in the slowly emptying than in the rapidly emptying stomach. However, a very considerable time is required for the reduction to alkalinity of quite a small amount of fluid, for, after complete emptying of the stomach, about half an hour is required to render the mucous membrane alkaline.

6. The importance of the cessation of gastric secretion for the neutralization process lies in the fact, that, so long as secretion continues, mucus produces no reduction of acidity unless very small amounts are being secreted. In this case the acidity falls according to the amount present and remains constantly at this level.

7. The acidity of a weak solution of HCl is reduced by a smaller amount than that of a strong solution. This is due to the irritant action of HCl, which produces a larger secretion of mucus according to its concentration.

8. The eventual end result of this neutralization process is always a fall to alkalinity, facilitated by emptying of the stomach, and rendered possible by the cessation of secretion of gastric juice. There is no mechanism regulating the acidity to any definite level, either by stoppage in the fall of acidity or output of acid to raise it.

9. The total Cl percentage is not regulated to any definite level. The final Cl percentage is not reached till the contents are alkaline, and it then stands at the variable level of that of mucus. There is no evidence whatever of the secretion of neutral chloride to raise the level of Cl percentage in the gastric contents, or of a diluting fluid other than mucus to lower it to any definite concentration. Unless the amount of gastric contents is small enough to be affected by mucus there is no attempt whatever to raise or lower the total Cl content or the acidity.

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THE HISTAMINE-HISTAMINASE SYSTEM IN THE ISOLATED PERFUSED KIDNEY-LUNG PREPARATION.

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In an earlier paper [MacGregor and Peat, 1931] we showed that stimulation of the sympathetic or parasympathetic nerve supply to the lungs in the intact cat produced no detectable changes in the histamine content of the lung, using the method of assay based upon the depression of a cat's blood-pressure. In a further endeavour to arrive at some conclusions regarding the significance of the presence in the body, in relatively large amounts, of such a potentially active substance as histamine, the investigation to be described was undertaken.

The discovery by Best [1929] of an enzyme system which specifically destroyed histamine and which was found to be present in many organs suggested to us the study of the relationship between this enzyme system and the naturally occurring histamine of the lung. Lung tissue itself contains this "histaminase," since Best [1929] found that on incubation of minced lung tissue in saline the naturally occurring histamine of the lung was rendered physiologically inert. Best and McHenry [1930] showed later that the kidney (at least in the dog) provided a much richer source of the enzyme. As a first step in our investigation we set out to find if a similar inactivation of the histamine occurred when the isolated but intact lung of the dog was perfused at 37° C. with defibrinated blood.

I. ISOLATED PERFUSED LUNG EXPERIMENTS.

The lungs were perfused with a Bayliss [Bayliss and Müller, 1928] pump under positive pressure ventilation; they lay on a gauze filter in a funnel which allowed the blood to drain freely from the open pulmonary veins. The pulmonary artery pressure was kept constant at 30 cm. of blood. The whole apparatus was enclosed in a thermostat. The dogs were anaesthetized with chloroform and ether with the exception of a few cases where nembutal was used. The lungs were washed through with defibrinated blood before being removed from the chest. In these and in

all the experiments to be described later the lung of one side only was perfused, that of the opposite side for the purpose of control being extracted with alcohol immediately on removal from the thorax.

The lung was weighed before and after the perfusion. There was invariably an increase in weight during the perfusion, and this increase we took as a measure of the extent of the vascular engorgement and oedema. The average increase in weight amounted to 80 p.c. All calculations of the histamine content were based on the weight of the lung before perfusion. In the extraction of the histamine from the various tissues we employed for lung and kidney the method in which the tissue is treated first with 96 p.c. and then with 60 p.c. alcohol, fats being removed from the extracts by shaking with ether [Best, Dale, Dudley and Thorpe, 1927]. A number of control experiments, in which histamine was added to the lung tissue before it was extracted, showed that the method involved a loss of about 25 p.c. of the histamine, but this loss was constant. In some cases we used the method favoured by Best and McHenry [1931] of disintegrating the tissue with acid. Comparative experiments showed that here the loss of histamine was less, but we prefer the former method, chiefly because of its convenience. Since we invariably worked up a control lung under the same conditions as the perfused lung, the absolute loss of histamine was of no moment. We found that the alcohol method could not be conveniently applied to blood, for which we used, instead, precipitation of the proteins and fats by means of Schenk's reagent. Here again control experiments showed that the loss of histamine occurring in the process was about 25 p.c. The histamine was assayed by the Burn and Dale [1926] method of depression of the blood-pressure of the atropinized cat. In a few cases the anæsthetic was ether alone, but in the majority we used medinal following ether induction.

We have tried other methods of assay, such as the chemical methods of Koessler and Hanke [1919] and of Zimmermann [1930] as well as those employing isolated artery strips, isolated rabbit's ear, isolated guinea-pig intestine as test objects, but we concluded that depression of the cat's blood-pressure was probably the most satisfactory available method. Nevertheless, it is not suitable for the determination of small changes in histamine content. In our previous paper we have quoted 20 p.c. as being the limit of our possible experimental error, but this value certainly varies with different animals. In many cases it is less than 20 p.c., but in a few, at least it is greater.

The average histamine content of the normal lung of the dog, calculated from twenty-four experiments, was found to be 16.9 mg./kg. This

value is lower than that found for cats (36.4 mg./kg.), but individual variations in the dog covered a much smaller range. The histamine content per unit weight of the control lung was taken as indicating the initial histamine content of the perfused lung. The results of five such experiments are summarized in Table I, which shows that perfusion of the intact isolated lung brings about neither inactivation of the histamine present in the lung nor its redistribution between the lung and the defibrinated blood.

TABLE I. Isolated perfused lung.

Exp.	Duration of perfusion, min.	Histamine in the lung, mg./kg.		Histamine change in lung, mg./kg.	Percentage histamine change in blood
		Before perfusion	After perfusion		
1	140	10.2	12.5	+2.3	0.0
2	180	16.7	16.7	0.0	0.0
3	180	25.0	25.0	0.0	0.0
4	180	12.5	12.5	0.0	0.0
5	180	10.0	10.0	0.0	0.0

These experiments also confirmed many others that we have made which all tend to show that defibrinated blood contains no detectable histamine as a normal constituent. On many occasions we have prepared extracts of blood by the alcohol-ether method and by precipitation with Schenk's reagent. We have used fresh whole blood, fresh defibrinated blood, and defibrinated blood which has been incubated for varying periods of time, without ever obtaining evidence of the presence of significant amounts of histamine. Most of these extracts showed a slight depressor action on the blood-pressure of the atropinized cat, equivalent (an average figure) to 1 : 4,000,000 histamine, agreeing with the figure previously given by Harris [1927] for human blood. But that this depressor substance in normal blood is histamine is rendered extremely doubtful by the conclusive demonstration by Zipf [1931] of the normal presence in fresh defibrinated blood of adenylic acid. We found that solutions of adenylic acid, in concentrations equivalent to those found by Zipf for blood, produced depression of the cat's blood-pressure of the same order as we obtained with the blood extracts.

II. PERFUSED KIDNEY-LUNG PREPARATION.

We are now in a position to investigate the effect of including the kidney in the circuit. The perfusion apparatus used for the isolated lung was extended to include the kidney in series. Means were provided for controlling and measuring the pressure in the renal artery and the blood flow through the kidney, the arrangement being somewhat similar to

that used by Hemingway [1931], Bayliss and Lundsgaard [1932]. The transfer of the kidney from the intact animal to the circuit was performed in as short a time as possible, which in the majority of experiments did not exceed 60 sec. We are indebted to Prof. E. B. Verney (personal communication) for the details of the operative technique.

TABLE II. Perfused kidney-lung preparation.

Exp.	Time of perfusion, min.	Histamine in lung, mg./kg.		Histamine in kidney, mg./kg.		Histamine change in lung, mg./kg.	Lung blood flow, c.c. per min.	Maximum kidney flow, c.c. per min.
		Before perfusion	After perfusion	Before perfusion	After perfusion			
1	240	20.0	33.0	N.T.	N.T.	+13.0	160	114
2	150	16.7	19.4	0.2	0.2	+2.7	240	60
3	180	12.0	12.0	0.5	0.4	0.0	250	155
4	130	19.6	21.8	0.17	0.21	+2.2	400	240
5	180	16.7	16.7	0.4	0.4	0.0	—	70
6	109	30.0	30.0	0.24	N.T.	0.0	250	100
7	132	10.0	10.0	0.2	0.2	0.0	270	112

N.T.=Not taken.

The results of seven experiments in which kidney and lung were perfused together are given by Table II. We estimated the histamine content of the remaining kidney for the purpose of control. The table shows that perfusion for periods varying from 2 to 4 hours does not induce the inactivation of any part of the naturally occurring histamine in the lung or kidney. This histamine is principally present in the lung. As already stated the blood contains no histamine, and columns 5 and 6 show that only a negligible amount is present in the kidney, before or after perfusion. This fact has already been noted [Best and McHenry, 1930]. One experiment only is not in keeping with this general conclusion. In Exp. 1 it is seen that a change occurs in the amount of histamine in the lung. The amount, 65 p.c. is outside our presumed limits of experimental error, but it is to be noted that it is an increase in the amount of histamine in the lung (and therefore in the system lung-kidney-blood) and not a decrease as one would expect. If this result were accepted, it would imply the actual synthesis of histamine during perfusion. It is, however, contradicted by the six experiments following it, and is neglected in drawing the above conclusions.

In view of the results given in Tables I and II it became necessary to test whether the kidney was actually functioning as an inactivator of histamine when the amine was perfused through it, using the isolated perfused kidney-lung preparation as in our previous experiments and adding histamine to the perfusate.

III. PERFUSED KIDNEY-LUNG PREPARATION WITH ADDED HISTAMINE.

Table III summarizes the relevant changes occurring when lung and kidney in series are perfused with defibrinated blood to which histamine (10 mg.) is added. It is clear that most of this added histamine was destroyed, although the perfusion never lasted more than $1\frac{1}{2}$ hours. Moreover, estimation of the histamine remaining in the blood at different times after its addition showed that the bulk of the histamine was inactivated within the first 5 min. after its addition.

TABLE III. Kidney-lung perfusion with added histamine (10 mg.).

Exp.	Time of perfusion after adding histamine, min.	Histamine in lung, mg./kg.		Histamine in kidney, mg./kg.		Percentage change of added histamine in blood	Amount of urine in c.c.	Absolute histamine content of urine (mg.)
		Before perfusion	After perfusion	Before perfusion	After perfusion			
1	28	20.0	30.1	0.0	1.0	-100.0	102	1.3
2	35	—	—	—	—	—	132	1.7
3	60	11.65	17.45	0.6	0.6	-100.0	82	0.7
4	85	10.0	13.3	—	—	-98.6	75	0.3

By these experiments we were convinced that the histaminase system of the kidney retained, under the conditions of our experiments, its full activity. The amount of histamine added to the blood was about ten times the amount that could have been furnished by lungs of the size we used. Evidently some cause other than that of inactivity of the kidney must be sought to explain the immobility of the natural histamine in the lung.

It will be seen from Table III that there is no loss in the histamine content of the lung. On the contrary, there is, if anything, in each case an increase, which as we shall see is comparable with the increase observed when the lung alone is perfused with blood containing histamine (Table IV). There is, moreover, no such storage of histamine in the kidney, whose histamine content remains negligible in amount. It would appear, therefore, that if any significance be attached to the small increase in histamine content of the lungs in these experiments, it must arise from the retention of the histamine added to the blood.

In one case only, in our kidney perfusions with untreated defibrinated blood, was the formation of urine observed, and in that case the blood was a mixture obtained from three different animals. In all the cases referred to in which urine was excreted by the kidney, this was attained by diluting the blood with approximately one-fifth of its volume of saline. We found

the addition of saline to have very little effect in increasing lung or kidney cedema. The alternative method of producing diuresis by addition of urea to the blood was not tried. In every case histamine, varying in amount from 0.3 to 1.7 mg., was excreted in the urine. The kidney therefore shows a twofold action on histamine, the greater part it inactivates in virtue of its histaminase, the remainder it excretes in the urine.

IV. ISOLATED PERFUSED LUNG WITH ADDED HISTAMINE.

In a further series of experiments the lung alone was perfused with defibrinated blood to which histamine was added. The results are summarized in Table IV, from which it is evident that there is a definite

TABLE IV. Isolated lung perfusion with histamine added.

Exp.	Pressure	Time of perfusion after addition of histamine, min.	Hista- mine added to the blood, mg.	Histamine in the lung, mg./kg.		Hista- mine change in lung, mg./kg.	Hista- mine change in blood, mg./kg.	Percentage histamine change in system
				Before perfusion	After perfusion			
1	50 mm. Hg	40	3.0	18.2	20.0	+1.8	-19.0	-11.7
2	30 "	50	3.1	20.0	29.3	+9.3	-45.1	-16.5
3	18 "	120	3.94	23.6	33.1	+9.4	-18.0	-2.6
4	12 "	215	2.04	26.7	26.7	0.0	-17.1	-9.5
5	35 cm. H ₂ O	120	4.00	11.7	20.0	+8.3	-51.1	-36.6
6	35 "	83	5.33	12.5	16.7	+4.2	-40.0	-27.4
7	35 "	90	5.33	16.7	25.0	+8.3	-28.4	-18.3

loss of added histamine from the system lung-blood on perfusion for $1\frac{1}{2}$ -2 hours. In the first four experiments the extent of the loss of this histamine comes within the limits of experimental error, but it is to be noted that in every case the figures in themselves indicate a loss and not a gain of histamine. Considering all seven experiments together, we feel justified in concluding that the lung perfused with defibrinated blood to which histamine has been added, destroys or inactivates part of this histamine, thus confirming and extending the results obtained by Best with minced lung tissues. A further conclusion to be drawn from Table IV is that, although histamine is destroyed, it appears to be only the histamine which has been added to the blood which is affected. In no case, as shown in column 7, is there a loss of histamine from the lung itself. On the contrary, except in Exp. 4, a small increase in the lung histamine is observed. In most cases the amount can be only partially accounted for by the accumulation of cedematous fluid containing histamine.

Acetylcholine.

In view of the fact that we had already tested [MacGregor and Peat, 1931] the effect of excitation of the parasympathetic nerves to the lungs on their histamine content, we considered it desirable to observe the influence of acetylcholine injections; we performed two experiments. As before the kidney-lung system was perfused with defibrinated blood. Eserine was first added to the blood to delay or inhibit the destruction of acetylcholine by the blood esterase [Engelhart and Loewi, 1930], followed about 10 min. later by acetylcholine bromide (4–5 mg.). Immediately on addition of the acetylcholine, in the two experiments the blood flow from the kidney rose from 100 and 86 c.c. per min. to 166 and 165 c.c. per min. respectively, while the kidney arterial pressure fell from 140 and 140 mm. Hg to 120 and 95 mm. Hg. This observation supports the findings of Hamet [1926] that acetylcholine dilates the kidney vessels. At the same time constriction in the lungs caused a decrease in flow from 176 and 272 c.c. per min. to 136 and 100 c.c. per min. in the two. In this connection Daly and Euler [1932] obtained vaso-constriction with acetylcholine in some of their isolated perfused lung preparations, in others vaso-dilatation occurred. Recovery of both lung and kidney was rapid, and at the end of the perfusion, although a trace of acetylcholine could be detected in the kidney in each case, there was no trace to be found in the lung. Examination of the lung, kidney and blood for histamine showed that blood and kidney contained none and that the amount in the lung (30.0 and 10.0 mg./kg. respectively) remained unchanged. Acetylcholine, therefore, does not cause the liberation of histamine from the lung in amounts determinable by our technique.

Pharmacological action of histamine on lung and kidney.

It may be of interest to record the effects we observed on adding histamine to the defibrinated blood, perfusing either lung alone, or lung and kidney together. As already pointed out, the pulmonary arterial pressure was constant throughout the experiments, and the renal arterial pressure was also maintained constant at 140 mm. Hg, except at those times when histamine was added to the circulation. In Table V are recorded the changes we observed in the renal artery pressure, the renal vein flow and the pulmonary vein flow on adding histamine to the circulation. These results indicate a definite vaso-constrictor action on the lung, while the vessels of the kidney are apparently dilated. Both effects disappear within 30 min.

TABLE V. Effect of histamine on the vessels of the lung and kidney.

Exp.	Histamine added, mg.	Kidney pressure (mm. Hg)		Kidney flow, c.c. per min.		Lung flow, c.c. per min.	
		Before histamine	After histamine	Before	After	Before	After
1	10.0	150	130	160	164	—	—
2	10.0	140	140	67	100	300	80
3	10.27	140	140	172	172	240	120
4	10.0	140	130	67	86	400	330
5	10.0	138	120	70	116	260	55

The increased resistance to flow through the lungs produced by histamine is in keeping with the observations of previous investigators [Dale and Laidlaw, 1910], but there appears to be no agreed opinion concerning its effect on the renal circulation. Dale and Laidlaw [1910] also observed a decrease of kidney volume in the intact animal, suggesting an active constriction. Manwaring, Monaco and Marino [1922] reported an increased resistance to flow in the isolated kidney of the dog. Dicker [1928] found a decreased venous outflow, while Morimoto [1928] thinks that histamine constricts the renal vessels. On the other hand Ganter and Schretzenmayr [1929] consider that histamine has a dilating effect on the capillaries of the intact kidney of the cat.

SUMMARY.

The relationship between the histaminase system of the lung and kidney of the dog and the naturally occurring histamine of the lung has been investigated by perfusion of the isolated organs with defibrinated blood.

1. Perfusion of the lung alone or of the lung and kidney in series has no effect on the gross amount of histamine in the lung. It is, however, expressly emphasized that the method of assay allows only of the estimation of large changes in the histamine content (20 p.c. or more) and says nothing about changes of a lower order than this.

2. When the lung alone is perfused with defibrinated blood to which histamine is added, part of this added histamine is inactivated, although the naturally occurring histamine in the lung is unaffected.

3. When the lung and kidney are perfused together with defibrinated blood containing histamine, most of the added histamine is rapidly destroyed, while some is excreted in the urine. Again, the natural histamine of the lung is untouched, despite the fact that when ten times the quantity of histamine in the lung is added to the blood, the greater part of this added histamine is inactivated within 5 min.

It is concluded therefore that within the limits of our experimental methods, as defined above, there appears to be no simple humoral connection in the perfused organs between the histamine of the lung and the histamine of either the lung itself or of the kidney.

We are greatly indebted to Prof. I. de Burgh Daly for his helpful criticism and advice.

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THE CENTRAL AND THE REFLEX MECHANISM OF PANTING.

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RICHET [1898], when describing the effect of exposure to heat on respiration in dogs, pointed out the striking difference between the behaviour of anæsthetized and non-anæsthetized animals. Non-anæsthetized dogs pant without a rise of body temperature, by simply being exposed to the sun for 2–10 min. To induce panting after administration of an anæsthetic (Richet used chloralose), however, the body temperature of the animal must be raised appreciably above normal. Richet assumes that in the first case the panting is based on a reflex, initiated by warming the terminations of the cutaneous nerves and of the fifth cranial nerve, while in the second panting is the direct result of raising the temperature of the brain centres.

A previous paper, published jointly with Prof. G. V. Anrep [Anrep and Hammouda, 1932], dealt with the respiratory changes which take place in the animal during panting. In the present communication it is proposed to study the nervous mechanism underlying the phenomenon of panting.

Richet thought that this peculiar form of respiratory activity was governed by the medulla. But subsequent researches on the Sherrington decerebrate preparation show that the medulla cannot be responsible for panting, since, in decerebrate dogs and cats, even at very high body temperatures, the highest respiratory rate does not exceed 60–70 per min.; decerebrate animals do not pant. Nikolaides and Dontas [1911] found that heat polypnœa cannot occur if the medulla is separated from the brain. They describe a heat polypnœa centre in the corpus striatum. Bazett and Penfield [1922] found in acute decerebrate cats and dogs a maximum rate of 60 at a temperature of 42.8° C., and Sherrington [1924] found in acute decerebrate cats and dogs a maximum rate of 75 with a rectal temperature of 41.1° C. On comparing the maximum rate of 60–75 per min. obtained in these decerebrate preparations with the

maximum of 300–400 observed in anæsthetized panting animals, one is led to the conclusion that the parts of the central nervous system responsible for the very high rates of respiration lie above the mid-brain.

THE "PANTING CENTRE."

The following experiments were carried out in order to try to localize more precisely the part of the central nervous system which is responsible for panting. It was hoped that by removal of various parts of the cerebral hemispheres it would be possible to locate, at least with some probability, that part of the brain which, when destroyed, interferes permanently with panting. Two different procedures were adopted. In the first, the section of the brain was performed beforehand (*i.e.* before panting); the dog was left to recover from the anæsthetic and from the immediate effect of the operation, and then warmed up. In the second, the brain was freely exposed on one or both sides, depending on the section to be performed; the animal was then warmed up until panting became definitely established, and the section of the brain was made during the progress of panting. When the first procedure was adopted, the dogs were operated upon under chloroform and ether anæsthesia; in the second case they were given chloralose intravenously (0.05–0.075 g. per kg.). All these animals were supplied with a tracheal cannula and had both carotid arteries tied. They were warmed up either in a hot bath at 40–42° C., or in an air thermostat by a method described in a previous communication [Anrep and Hammouda, 1932]. The respiratory rate was recorded by a stethograph. After the end of an experiment, the brain was hardened *in situ* with formalin; it was then removed from the skull and carefully examined to determine the extent of the injury¹. The operations on the brain were all bilateral. The experiments with the section of the brain at different levels can be classified under the following four headings:

- (1) Removal of the cortex with preservation of the basal ganglia.
- (2) Removal of the two hemispheres leaving the mid-brain intact.
- (3) Removal of the cortex and of the corpora striata without damaging the optic thalami.
- (4) Local injuries to the optic thalami.

Fig. 1 shows the levels at which these sections were made.

In the first group, the animals were decorticated on both sides by scooping out the brain substance, care being taken not to injure the basal

¹ I am greatly indebted to Dr B. Boulgakow, of the Anatomy Department, for the help he gave me by making the post-mortem examinations of all the brains.

ganglia; the cavity of the skull was then lightly packed with cotton-wool soaked in warm saline. The post-mortem examinations of these brains showed that the basal ganglia were completely intact, and that practically the whole cortex had been removed, *i.e.* only occasionally had small scraps of the cortex been left. All these animals reacted to the rise of body temperature by panting as vigorously as normal animals. This was observed in dogs in which the cortex was removed beforehand, as well as in dogs which were decorticated during the progress of panting. In the latter case, the panting respiration was slightly disturbed during the

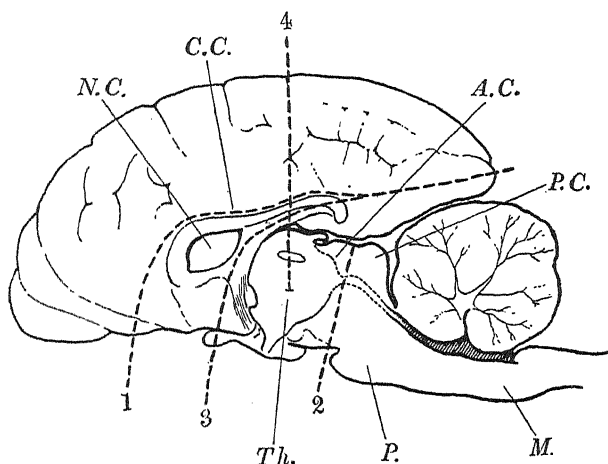


Fig. 1. Sagittal section of dog's brain, demonstrating the operations (1, 2, 3, 4) performed. *C.C.* corpus callosum; *Th.* optic thalamus; *N.C.* nucleus caudatus; *A.C.* anterior colliculus; *P.C.* posterior colliculus; *P.* pons; *M.* medulla. Further description is given in the text.

actual surgical interference, but afterwards it continued without interruption. Panting as a response to raising the body temperature is thus shown to be independent of the cortex.

In the second group of dogs, the section was made at the level of the anterior colliculi. The post-mortem examinations showed that the brain stem was completely severed in all cases; no parts of the optic thalami had been left behind. In a few experiments it was also found that the anterior colliculi had been destroyed, but the posterior colliculi remained uninjured in every experiment. During the operation on non-panting dogs, the respiration stopped for a moment and then resumed its original rhythm. On warming up these animals, the respiration accelerated only to a maximum of 40–60 per min. with a rise of rectal temperature to

42.0° C. The higher respiratory rates were observed in those dogs in which the plane of section was more anterior, thus causing less disturbance to the mid-brain. When the decerebration was made in a panting animal, the respiration stopped during the section and then resumed at a slow rate, which gradually rose to a maximum of 40–60 per min. In two of these experiments, the temperature was raised until the dogs succumbed to heat; panting, however, never took place. Before death, the respiration became slow and was occasionally interrupted by gasps. In the other experiments, the animals were allowed to cool. It was noticed that in these cases the temperature dropped considerably more rapidly than in normal animals; it fell in a comparatively short time to 33–31° C. The results obtained on decerebrate animals confirm the observations of Bazett and Penfield [1922] and of Sherrington [1924] that the maximum rate obtained in such preparations does not exceed 75 per min., a rate which is far below even the slowest rate obtained in panting. These experiments also make it clear that the part of the central nervous system which is responsible for panting lies somewhere between the cortex and the mid-brain, *i.e.* in the basal ganglia.

In order to find out which of these ganglia is concerned, experiments were made in which the corpus striatum was destroyed with the least possible damage to the optic thalamus. A slightly curved spatula, 1.3 cm. in breadth, was employed for this purpose. After freely opening the skull on both sides, having previously measured the exact distances by comparison with a preserved brain of the same size, the spatula was introduced through the occipital lobe. It was held so that its lateral edge was somewhat lower than the other edge. The spatula was pushed into the brain substance in this slanting position until the anterior limits of the thalami were passed, and then it was pressed downwards to the base of the skull. The operation was repeated on the other side of the brain. As a result of this operation it was hoped to destroy the greater part of the corpora striata without injuring the optic thalami. Three experiments were successfully performed. The post-mortem examinations of these animals, made by serial frontal sections, showed that the lenticular and the caudate nuclei as well as the internal and the external capsulae were in every case destroyed almost completely; the colliculi and the mid-brain were intact. The optic thalami were intact in two cases, while in the third their anterior parts were slightly bruised. Of the two dogs with the intact thalami, one was operated upon during panting; its respiratory rate was 200 per min. and its rectal temperature 41.4° C. The respiration was momentarily interrupted during the sections, but

soon after the operation the dog was breathing at 60 per min., and within 1 min. it was again panting at the rate of 200. The animal was then cooled; at a rectal temperature of 40.7° C. panting disappeared, the respiratory rate being 40 per min. The dog was cooled down to 37° C. and, on rewarming, it began to pant at the rate of 200–250 per min. when the rectal temperature reached 40° C. The second dog, which was operated upon 3 hours before the exposure to heat, panted at 120 per min. with a rectal temperature of 40.5° C., and at 250 per min. with a temperature of 41° C. In the third dog also the operation was performed 3 hours before warming. On exposing this dog to heat, its rectal temperature gradually rose to 42.5° C., and the respiration accelerated with the rise of temperature to 58 per min.; the breathing then became irregular and was interrupted by deep gasps. No panting was observed. The dog was cooled and then warmed up to 43° C. with exactly the same result. This was the dog in which the thalami were found to be injured.

These experiments with acute spino-thalamic animals show that the development and the extent of panting is of the same character as that observed in normal animals, and that the least injury to the thalami abolishes panting and makes the animal behave as if it were decerebrated. As in all the three experiments the corpora striata were almost completely destroyed one is led to the conclusion that these ganglia are not concerned in panting. The experiments strongly suggest that the part of the central nervous system on which panting depends, or to give it a name the "*panting-centre*," is located in the optic thalamus. This conclusion is supported by experiments in which four or five serial frontal sections of the brain were made during the progress of panting, and by experiments in which localized injuries to the thalami were inflicted. The roof of the skull was completely removed, the longitudinal sinus was removed between ligatures, and the brain was exposed. The dog was then kept immersed up to its neck in a hot bath, until panting became well established. With a sharp flat spatula, frontal sections were made across the hemispheres down to the base of the skull. The first section was made through the anterior parts of the frontal lobes; the panting was undisturbed. After an interval of a few minutes, a new section was made about 1 cm. behind the preceding section; this also had no effect. Sections were continued at short distances behind each other until panting disappeared, after which no more sections were made. In the three experiments in which this was carried out, panting remained unaffected until a section passed through the optic thalami. It should be noted that a section which passes just in front of the thalamus definitely

cuts through the heads of the caudate nuclei. Such a section, however, has no permanent effect on panting.

Localized injuries to the thalami were made by means of a small metal tube. During the progress of panting, the tube was introduced between the hemispheres, through the corpus callosum and the fornix into the third ventricle. This in itself had no effect on panting. The thalami were then injured on both sides, and the extent of the injury was determined in the post-mortem examination of the brain. Four such experiments were made. During the actual injury, the respiration either stopped or showed considerable irregularities. After removal of the metal tube these irregularities persisted for a short time, but soon the respiration assumed a regular rate which varied in different experiments from 60 to 100 per min. When the warming of these animals was continued, the respiration rate did not increase any further. In a few experiments, the damage of the thalami was made by cauterization with a fine electro-cautery, which was passed into the third ventricle in the same manner as the metal tube. It was usually found that the greater the damage to the optic thalami, the slower was the respiration; but even comparatively small amounts of damage may considerably reduce the rate of panting. Fig. 2 shows the extent of the damage

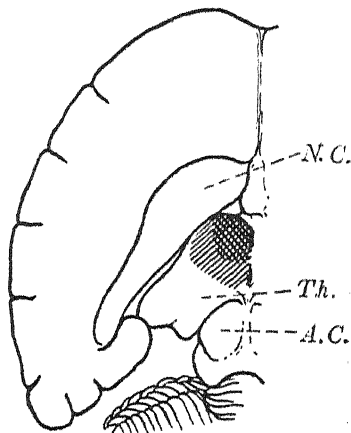


Fig. 2. Diagram to illustrate the localized injuries to the thalamus, as found in the post-mortem performed after the two experiments referred to in the text. The extent of the injuries in the two experiments is represented by the boundaries of the two differently shaded areas. In each case the injury was almost identical on both sides of the brain. *Th.* optic thalamus; *N.C.* caudate nucleus; *A.C.* anterior colliculus.

to the optic thalami in two cases. The results of serial sections of the brain and of local injuries to the thalami thus definitely indicate that panting is dependent on the integrity of the optic thalami.

Abundant evidence is given in the literature that the temperature control of the animal is situated above the level of the mid-brain. Barbour [1912] locates the thermotaxic centre in the corpus striatum, but Cloetta and Waser [1914] do not support this view. Isenschmid and Kretzl [1912], Isenschmid and Schnitzler [1914], Leschke [1913], de Barenne [1920], and Bazett and Penfield [1922] are of the opinion that the actual position of the centre which controls body tem-

perature is probably in the thalamus, hypothalamus or tuber cinereum. Panting is a specialized mechanism of regulation of the body temperature. It is therefore not surprising that the centre for panting should be localized in the same region as the other temperature controlling centres.

THE CENTRAL MECHANISM OF PANTING.

Raising the body temperature of an anaesthetized dog leads to panting. Richet ascribed this to a direct stimulation of the respiratory centre by heat, but he advanced no conclusive evidence in favour of this view. Richet refers to the experiments of some older observers who found that panting can be produced by warming the carotid blood supply. These observations were confirmed by Kahn [1904] and by Heymans and Heymans [1926]. Bazett [1927], however, rightly remarks that the experiments in which the carotid blood stream is warmed cannot be regarded as conclusive evidence of the direct stimulation of the centres by heat, unless precautions are taken to exclude the influence of sensory impulses through the cranial nerves. This criticism assumes a special importance since Richet points out that the application of heat to the terminations of the fifth cranial nerve plays an important part in the production of panting in non-anaesthetized animals.

The following experiments were performed in order to determine whether the panting produced by warming the carotid blood is really due to a direct heat effect on the centre. The vertebral arteries were tied and the animal was heparinized. The central ends of both common carotid arteries were connected with a glass spiral which was immersed in a water bath. The blood flowing through the spiral (where it could be warmed or cooled) was led into the peripheral ends of the common or of the internal carotid arteries. The temperature of the blood was measured close to the peripheral ends of the arteries. The venous blood was allowed to return to the heart of the animal. The temperature of the body, which was measured in the rectum, was kept as constant as possible. On warming the carotid blood, panting could be obtained with great ease. No difference was observed between the panting of animals which were perfused through the internal or the common carotid arteries, showing that the presence of the carotid sinus plays no rôle in panting. This was confirmed by two experiments in which the whole animal was warmed after extirpation of the carotid sinus on both sides. The panting presented no difference from that in animals with intact carotid sinus. These results are in agreement with the observations of Heymans and Heymans [1926] and Tournade and Malméjac [1930].

In the experiments in which the head is perfused through the internal carotid arteries (the vertebrals are tied) besides the exclusion of the carotid sinus, the blood supply to the peripheral structures of the head and therefore to the terminations of the fifth cranial nerve must be considerably reduced. In order to test whether panting may be caused by warming these or other sensory terminations in the head, the following additional observations were made. The head of an animal, while being perfused through the internal carotid arteries by a blood supply sufficiently warmed to cause panting, was surrounded by ice, and the nasal and the buccal cavities were continually irrigated with cold saline. The panting, however, remained unchanged. Further, it may be added that the removal of the whole skin of the head also fails to affect panting. These experiments show that at least the external sensory terminations of the head play no rôle in the causation of panting in response to raising the temperature of the blood. It has already been mentioned that extensively opening the skull and the meninges, which necessarily prevents their warming, does not modify panting. Unless there are specialized heat receptors in the meninges at the base of the brain or in the cerebral blood vessels, all the evidence points to panting as being produced by an entirely central mechanism, *i.e.* by the direct effect of heat on the "panting centre."

In several experiments an attempt was made to produce panting by direct application of heat to the optic thalami. A narrow Kronecker's cannula was introduced into the third ventricle by the technique described above. Great care was taken to fix the cannula in such a position that it should not exert pressure upon the thalami. One branch of the cannula was connected with a reservoir containing warm saline, and the other was connected with a rubber tube which served as an outlet. The third ventricle was perfused at a low pressure with warm saline. The temperature was measured in the reservoir and in the inlet branch of the cannula. During the irrigation of the ventricle with saline at 38° C., the respiration was regular throughout. On raising the temperature of the perfusing fluid in the inlet cannula to 42° C., it was observed in some cases that the animal started to pant. The panting could easily be stopped by dropping the temperature of the perfusing fluid. The panting rate was in no case very high, but, as can be shown by a method described below, it was a genuine panting and not a simple acceleration of the ordinary respiration. Warming of the thalami by this method would obviously raise the temperature chiefly at the surface of the thalami. without much affecting the rest of the thalamus; moreover, manipu-

lations in this region would invariably cause some disturbance or damage. This is probably the reason why local warming of the thalami never leads to such high rates of panting as are observed in the case of warming the carotid blood or the whole animal. In fact, in experiments in which the introduction of the cannula caused definite damage to the thalami, perfusion with hot saline failed to induce panting. A macroscopic examination of the thalami was made after the end of each experiment. It must be specially emphasized that experiments with the direct warming of the thalamus are not invariably successful, but those which were successful leave no doubt about their result. It is important to choose a suitable anaesthetic. In chloralosed animals, the effect was not obtained. Urethane or alcohol anaesthesia was found to be the best. It is of interest to mention that in some experiments a gentle mechanical stimulation of the mesial surfaces of the thalami frequently produced panting, but the rate did not exceed 120–130 per min. The following is an experiment with direct warming of the thalami.

Dog 6 kg., morphine 25 mg., 5 c.c. of 42 p.c. alcohol intravenously. The injection of alcohol was repeated in 1 c.c. doses during the experiment. The roof of the skull was removed, the meninges opened and the longitudinal sinus cut out between ligatures. All bleeding points were stopped. The respiratory rate was 22 per min. 30 min. after opening the skull, a thin Kronecker's cannula was introduced into the third ventricle. The respiration at first increased to 34, and then returned to 22 per min. Saline at 38° C. was now passed through the cannula into the ventricle; the outflowing fluid at first contained traces of blood, but in a few minutes it became clear. The respiratory rate fluctuated between 20 and 22. The perfusion was now changed to saline at 42° C. (registered in the inlet of the cannula). The following were the respiratory rates during successive 20 sec., starting from the moment of the irrigation with hot saline; 20, 60, 80, 80, 100, 100, 120, 120, 120, 130, 130, 130 per min. The ventricle was now perfused with cold saline at 25° C. The rates were 100, 80, 60, 60, 50, 48, 36, 22, 18 per min.

This experiment was repeated several times on the same animal with the same result. The cannula was then introduced more deeply into the third ventricle, and moved in a frontal plane so as to produce localized damage to the thalami. The irrigation was continued. The respiration, after some irregularities, returned to 18 per min. and remained at this rate. Irrigation of the ventricle with hot and cold saline now produced no change in the respiratory rate. The rectal temperature of the animal was kept between 37·0 and 37·5° C. throughout the experiment. The post-mortem examination showed extensive damage of the mesial parts of both thalami; the corpora striata and the anterior colliculi were intact.

THE REFLEX MECHANISM OF PANTING.

The experiments with perfusion of the carotid arteries, and with the direct application of heat to the optic thalamus, are strong evidence in favour of the existence of a central mechanism of panting. Richet assumed that this was the only mechanism on which panting depends, in animals which have been subjected to anæsthesia. He did not, however, perform any experiments in order to verify this assumption. The question whether there is also a reflex mechanism, which co-operates with the central one, still therefore remains unanswered. In other words, we do not know whether warming of some sensory terminations of the body in an anæsthetized animal may evoke reflex panting. It would be legitimate to suppose that the skin of the animal would be the most likely organ to act as a receptor in such a reflex. This is, however, not the case, as a dog will pant when its body is warmed even after the removal of the whole skin. The body temperature at which panting develops does not differ from the panting temperature of a dog whose skin is intact. The rate of panting is also of the same order. The following experiment is an example.

Dog 3.5 kg., morphine 20 mg., chloralose 0.25 g. On exposure to heat in a water bath at 42° C., the dog began to pant at a rectal temperature of 40.0° C. When its temperature rose to 40.5° C., the dog panted at a regular rate of 200. The dog was cooled and the anæsthesia was deepened by administration of chloroform and ether mixture. The whole skin of the animal from the tip of the nose to the end of the tail was then removed, care being taken to stop all bleeding points. The volatile anæsthetic was discontinued, and the animal was at once placed in a saline bath at 38° C. and allowed to rest for 45 min., after which the temperature of the bath was raised to 41° C. The dog, as before, started panting at a rectal temperature of 40.0° C. and soon reached a rate of 230 per min. with a rectal temperature of 40.2° C.

This experiment definitely shows that the skin plays no rôle in panting produced in response to the warming of the whole body. The following experiments, in which the carotid blood stream was warmed or cooled independently of the changes of the body temperature, demonstrate further that the warming of the internal organs of the dog also does not cause panting.

The blood stream in the common carotid arteries was passed through a water bath, in the same manner as in the experiments referred to before, while the body of the dog was placed in an air thermostat. The temperature of the head was measured by a thermometer placed deeply under the tongue, and the temperature of the body by a thermometer in the rectum. In the first part of the experiment, the body and the carotid blood were warmed at the same rate. The animal, which breathed at the rate of 18-22 per min., gradually increased the respiration to 33 per min. At a temperature of 41° C. in the mouth

and rectum the animal abruptly began to pant, increasing its panting within 4 min. to 240 per min. The head was now gradually cooled to $38.5^{\circ}\text{C}.$, but the body was warmed still further until the rectal temperature reached $42.5^{\circ}\text{C}.$ In spite of this, the respiration dropped to 26 per min. On rewarming the carotid blood to $41^{\circ}\text{C}.$, the panting returned to 250 per min. The head was then kept at $41^{\circ}\text{C}.$, while the whole body was gradually cooled to $37.5^{\circ}\text{C}.$ The panting rate did not change; it fluctuated between 230 and 250 per min.

A second experiment was started by warming the body alone, the mouth temperature being kept at $37.5^{\circ}\text{C}.$ The rectal temperature was gradually raised to $41.5^{\circ}\text{C}.$ but the respiration remained unchanged; it varied between 26 and 30 per min. The body was then cooled to $37^{\circ}\text{C}.$ while the head was gradually warmed; at a mouth temperature of $39^{\circ}\text{C}.$, the respiratory rate became 200 and, at $39.5^{\circ}\text{C}.$, 290 per min.

These experiments give strong support to Richet's statement that in chloralosed dogs panting is entirely of central origin. Reflex panting could not be observed in anæsthetized dogs.

RESPIRATORY REFLEXES DURING PANTING.

Richet [1898] refers to Goldstein and Sihler as having been the first to show that section of the vagi does not affect panting. This observation was verified and extended in a previous communication [Anrep and Hammouda, 1932]. It can now be added that in panting, besides playing no rôle, the vagus nerve also loses its normal controlling action of the respiratory movements. The reflex arrest of the respiration in response to inflation of the lung [Hering and Breuer, 1868] is absent during panting (see Figs. 3 and 4). This could be seen in every experiment. It is so constant a phenomenon that it is convenient to take the disappearance of the reflex to inflation as an arbitrary criterion for distinguishing between panting and fast rates of normal respiration. Usually the conspicuous difference in the rate between ordinary respiration and panting leaves no doubt as to which type of respiration one is dealing with. But in some cases, especially in deeply anæsthetized animals in which the panting rate may not exceed about 120 per min., the disappearance of the reflex is of considerable help. The disappearance of the pulmonary vagal reflex is not abrupt. During the period of transition to panting, inflation of the lungs can still produce a brief arrest or slowing down of the respiration. This arrest, however, differs from that observed in the case of ordinary respiration by being considerably delayed in its appearance. The normal respiration is stopped at once as soon as the lungs are inflated, while in panting there are always a few respiratory movements before the arrest; these respiratory movements are frequently unchanged in rate (see Fig. 4*a* and *b*). As panting develops, progressively larger degrees of inflation are required in order to produce this delayed

pause. Finally, as panting becomes established, inflation of the lungs ceases to have any inhibitory effect on respiration. In several experiments, an acceleration of the respiration was noticed during the inflation, instead of an inhibition. Although gradual, the disappearance of this Hering-Breuer reflex occurs rapidly. In most experiments, the transition period lasts approximately the same length of time as it takes the dog to develop the fast rate of respiration which is definitely recognized as panting. This may require from 10–15 sec. to 1–2 min.

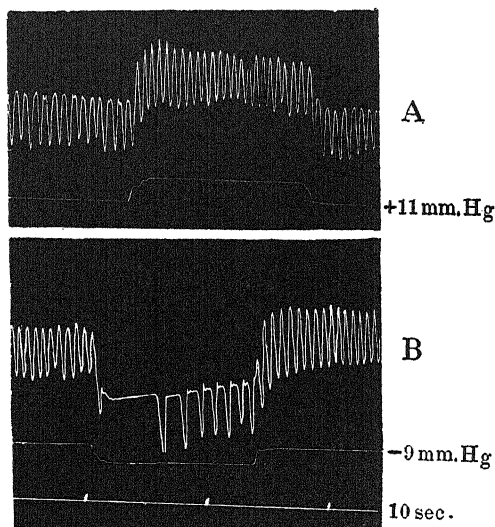


Fig. 3. Effect of deflation and inflation of the lungs during the transition from normal respiration to panting. The lungs were deflated and inflated by positive and negative pressures applied to the animal which was enclosed in a box. *A*. Effect of deflation by +11 mm. Hg at a respiratory rate of 80 per min. The respiration accelerated to 100 per min. *B*. Effect of inflation by -9 mm. Hg at a respiratory rate of 95 per min. Read from left to right. Time in 10 sec.

In the great majority of the experiments (over 200 dogs), the respiratory rate of 120 per min. could be recognized as an unquestionably established panting. Rates between 75 and 120 showed a progressive diminution, but not a complete absence, of the respiratory response to inflation of the lungs.

The usual acceleration of the respiration in response to a deflation of the lungs [Hammouda and Wilson, 1932] could not be observed during panting. For instance, in one experiment, just before the development of panting, the animal's respiration, which was 54 per min., accelerated

to 108 when its chest was exposed to an outside positive pressure of 13 mm. Hg. A few minutes later the animal started panting at the rate of 120. The compression of the chest by 16 mm. Hg air pressure did not

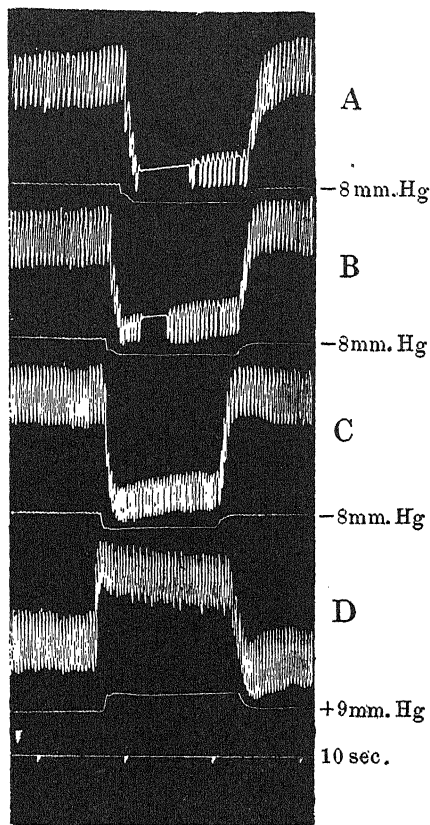


Fig. 4. Effect of inflation and deflation of the lungs during panting, from the same dog as Fig. 3. *A*, *B* and *C* show the effect of inflation (-8 mm. Hg); the respiratory rates were 114, 120 and 150 respectively. In *D*, deflation ($+9$ mm. Hg) did not change the rate of 162 per min. Time in 10 sec. Read from left to right. The line under each respiratory record shows the pressure change in the box enclosing the animal. The respiration was recorded by means of a bellows recorder which was attached to a closed respiratory system of 20 litres capacity, connected with the trachea of the animal. In between the records, the trachea of the dog was opened to the air.

change the rate. It is obvious that these experiments are not as conclusive as those with the inflation of the lungs, since it is always possible to assume that the respiration was already too fast to be further accelerated by the deflation of the lungs.

The absence of the Hering-Breuer reflex is not due to a possible effect of heat upon the terminations of the vagus nerve in the lungs. This is seen from experiments in which panting was produced by warming the carotid blood while the body temperature was not raised; in this case inflation and deflation of the lungs also failed to change the rate of panting.

PANTING IN THE NORMAL DOG.

On comparing panting in anæsthetized and non-anæsthetized animals, one is at once struck with the fact that, while in the former panting is undoubtedly of a central nature, in the latter it exhibits all the properties of a reflex. This contrast is still further accentuated by the fact that, under ordinary experimental conditions, it seems impossible to obtain reflex panting in the anæsthetized dog, while in the normal dog it takes place with the greatest ease. A dog which is exposed to the sun begins to pant in a few minutes. Careful measurements of the rectal temperature in most cases show an initial rise of about 0.1°C . This rise of temperature cannot, however, be the cause of panting since, as soon as panting starts, the temperature drops below the prepanting level, usually not less than $0.3\text{--}0.5^{\circ}\text{C}$. and sometimes as much as a whole degree. This was observed by Richet and used by him as a proof of the purely reflex character of panting in non-anæsthetized animals.

It can easily be shown that not only exposure of the whole animal to the sun but also exposure of parts of its surface leads to panting. For instance on one occasion, when the head of a dog was exposed to the sun while its body was kept in the shade, panting started in $5\frac{1}{2}$ min. On exposing the hind quarters it started in 6 min., while exposure of the whole animal evoked panting in 3 min. The temperature of the sun in this case was only 46°C .¹ On another occasion on exposing the head alone, when the temperature in the sun was 52°C ., panting started in $5\frac{1}{2}$ min., while on exposing the body with the head kept in the shade it started in 30 sec. At a still higher temperature (65°C .), the dog may start panting almost at once. No precise determinations were made of the minimal surface which had to be exposed to the sun in order to obtain panting. It became evident, however, from the observations made that the exposure of the head does not play any special rôle in com-

¹ The monthly mean temperature in Cairo, taken with the black bulb thermometer, at 2 p.m. in the sun, varies between 51°C . in December and 68°C . from June to October. The relative humidity is 62–66 p.c. during winter and 45–55 p.c. from February to September.

parison with the rest of the body. The rectal temperature of these animals either remained unchanged or rose not more than 0.1°C .

Richet states that, in the conscious animal, panting does not take place at all if some obstruction to respiration is introduced. For instance, he quotes as an example that a muzzled dog does not pant. As a consequence of this, its body temperature rises in a short time. This inhibitory action (of closing the mouth) on panting is, however, far from being so simple. Muzzling a dog for the first time may delay the appearance of panting for half an hour or more, and the animal's temperature may rise quite appreciably; but in the end panting invariably starts. Placing the muzzle on an already panting animal may stop panting completely for many minutes. It is of importance that if the muzzle is used for the second or third time it loses its inhibitory effect. An animal which is accustomed to the muzzle begins to pant almost as soon as without the muzzle; muzzling such an animal while panting is in progress does not stop panting any more. There is, however, one effect of closure of the mouth which remains. With the muzzle, the panting is slower and deeper than without. For example, placing the muzzle on an animal, which was already comparatively accustomed to it, reduced the panting rate from 300 to 220 per min.; on removing the muzzle, it returned to 300. It has not yet been found whether this effect is constant or shows signs of diminution on repetition of the experiments. The abolition of panting observed during the first application of the muzzle is not due to closure of the mouth. In fact, any other discomfort may also produce arrest of panting. A slap, rapid lifting of the animal, or the application of any other stimulus, if sufficiently strong, abolishes panting for some time. Auditory and strong visual stimuli may do the same. All these stimuli are alike in that their repetition rapidly ceases to have an effect. In this respect these stimuli show a striking similarity to the well-known phenomenon of external inhibition of conditioned reflexes [Pavlov, 1927].

It is not only external stimuli which may stop panting. If the animal is thirsty or hungry, if its bladder or bowels are full, exposure to sun does not cause panting until its rectal temperature rises appreciably above normal, which usually takes 30–45 min. at an external temperature in the sun of $37\text{--}45^{\circ}\text{C}$. When the cause of the discomfort is removed, exposure to the sun again produces panting within a very short time, and without a rise of body temperature. In many cases the disturbing cause could not be traced.

The similarity between panting in the non-anæsthetized animal with conditioned reflexes was so striking that it seemed probable that what is

known as reflex panting is really a conditioned reflex. It is as yet impossible to state whether every case of reflex panting, occurring without or with a minimal rise of body temperature, is conditioned panting. But the failure to obtain panting in anaesthetized animals lends support to this supposition.

Conditioned panting can be established with great ease. In several animals this was done in the following manner. A dog, which was quite new to this type of experiment but accustomed to the laboratory, was placed in a specially constructed large box, where it was subjected to a high temperature. The box was well ventilated. Ten shielded carbon-filament lamps were used as a source of heat, and the temperature of the box was kept at 45° C. In every case the respiration was recorded graphically by means of a stethograph. The development of conditioned panting will be described in relation to one dog only. On several days before the experiment the dog was placed in the unheated box (20–22° C.). In no case did it start panting. The dog was then placed in the heated box once or twice a day. The latent period during the first six exposures varied between 8 and 4 min., gradually becoming shorter. On the third week of working with this animal, the latent period shortened to between 30 sec. and 2 min. On several occasions the dog panted as soon as it was placed in the box. The experiment would usually be terminated a few minutes after the onset of panting. The maximum panting rates were between 150 and 200 per min. After forty-one exposures, the dog was placed in the box which was not heated (20° C.). The respiration of the animal was 22 per min. Thirty seconds after the introduction into the cold box the respiration increased to 60–80, and in the following minute to 120. The second test was made the next week, after a few more reinforcements of the reflex. The latent period was again 30 sec. During several trials, in between which the reflex was steadily reinforced every day, the latent period was found to vary between 15 and 30 sec. The maximum rate of this conditioned panting was between 120 and 150 per min.

Conditioned panting undergoes experimental extinction as can be seen from the following experiment. On the third month of the development of the reflex, the dog was placed in the cold box and then removed about 30 sec. after the onset of panting. After an interval of 5 min. the dog was again introduced into the box; this time the latent period was considerably longer. The procedure was repeated until panting could not be obtained, which happened on the fifth introduction into the cold box.

The phenomenon of external inhibition could also be easily observed. This effect on conditioned panting is remarkably similar to the effect of extraneous stimuli on a dog which pants under ordinary circumstances when exposed to the sun.

SUMMARY.

1. Serial sections of the brain and localized injuries to the optic thalami made in acute experiments demonstrate that panting takes place only when the optic thalami are intact.

2. Removal of the cortex and almost complete destruction of the corpora striata does not abolish panting.

3. Panting observed in anæsthetized animals on raising the body temperature is due to the direct effect of heat upon the optic thalami. It is not due to any reflexes in response to raising the temperature of sensory nerve endings in the head.

4. Heat directly applied to the third ventricle was, in several experiments, successful in evoking panting. It failed to have this effect after injury to the thalami.

5. The inhibition of the respiratory movements caused by the inflation of the lungs, and the acceleration of the respiration caused by deflation, is not observed during panting.

6. The experiments failed to demonstrate the existence of any special receptors in the body which would reflexly evoke panting in response to a rise in body temperature.

7. The normal non-anæsthetized animal pants reflexly without or with a very small initial rise of temperature. This panting exhibits the characters of a conditioned reflex. Conditioned panting can easily be established experimentally.

8. Spontaneous panting on exposure to the sun and conditioned panting are similar in that they are both subject to external inhibition. Conditioned panting undergoes experimental extinction.

9. It is suggested that what is known as reflex panting is a conditioned reflex which is developed, not on the bases of an unconditioned reflex, but on the bases of the purely central effect of heat. In this respect it would be similar to the conditioned reflexes established as the result of injection of various centrally acting drugs.

The research described in this communication was inspired by some observations made during my conjoint work with my teacher, Prof. W. H. Wilson. I wish to express my grateful thanks for his guidance and

encouragement. The actual experiments were made under the direction of Prof. G. V. Anrep, F.R.S., to whom I am grateful for his constant advice and criticism.

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LAPICQUE'S THEORY OF CURARIZATION.

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CURARE will bring about a condition in which an impulse will not pass from nerve to muscle though each tissue will still conduct. This condition is called curarization and the term is extended to include similar states brought about by various drugs and fatigue, etc. Thus "curarization" as used in the present paper will not necessarily signify that the conduction block was produced by curare, but it will simply mean that indirect excitation of muscle has been abolished though nerve and muscle are individually still irritable and able to conduct.

Claude Bernard's analysis of the action of curare is classical, and until the beginning of the present century it was universally accepted that there was a special substance intermediate between muscle and nerve which was particularly susceptible to curare and other drugs. In 1906, however, Lapique put forward an alternative hypothesis which has subsequently gained great support, so that at present it is very widely held. According to Lapique the strength-duration curve of a muscle is normally identical in time relations with that of its motor nerve, and this "isochronism" is considered a necessary condition for the propagation of impulses from one tissue to another. If a drug or other agent causes the chronaxie of the two tissues to diverge by more than a ratio of 2:1 then conduction between the two is supposed to become impossible. This theory has not been developed deductively from the physics of the conduction of impulses, and no great weight has been attached to theoretical attempts to explain why conduction should fail when two tissues develop a heterochronism in the ratio 2:1. Essentially, the theory is the expression of experimental observation, and hence its validity rests upon the soundness of the experiments adduced to support it. These experiments, which appear to be of three kinds, are each open to certain objections,

and the aim of the present paper is to provide a critical and experimental study of them in order better to appreciate the basis of Lapique's theory.

Within the last two years Lapique [1931] has insisted that the name "Chronaxie" be restricted to very special conditions, hence in this paper Lucas's term "Excitation Time" will be used, for in this way no theoretical distinctions will be introduced into a purely experimental study. The question will be resumed in the Discussion.

LAPICQUE'S FIRST ARGUMENT.

Historical.

At first sight it would seem that experimentally there could be no doubt as to whether the curarization was or was not accompanied by a heterochronism of more than 2:1. Curare is admitted on all hands not to affect nerve, thus it merely remains to excite muscle directly and compare the strength-duration curves before and after curarization. This is in fact what Lapique did, but the results are complicated by the difficulty of knowing whether the tissue initially excited was the muscle or the intramuscular nerve twigs. Lapique used the gastrocnemius muscle which has no part that can safely be regarded as nerve-free, he took no special precautions to avoid stimulating nerves, he obtained strength-duration curves identical with nerve, and found a sudden change in the curve at the moment when indirect excitation was abolished [Lapique, 1926, p. 245].

It is difficult to reconcile the statement at the bottom of p. 244 with that on the middle of p. 245 referred to above. The first reads "On n'obtient ni changement brusque, montrant qu'on passe de l'excitabilité nerveuse à l'excitabilité musculaire, ni...": the second "...il est presque impossible de saisir un intermédiaire entre la valeur primitive de la chronaxie, et la valeur double." Then follows a statement of Lapique's opinion that he was initially stimulating intramuscular nerves.

It therefore appears very likely that initially Lapique was measuring the strength-duration curve of nerve, and his experiments give no direct information as to whether there is normally isochronism between muscle and its nerve or not. This difficulty is well appreciated by Lapique, who attempts to support his case by the following argument.

After indirect excitation has been abolished there is no question but that muscle is the tissue investigated. But Lapique observes that at the moment when indirect excitation fails the excitation time of muscle is double that of nerve, and owing to the continued action of the drug it gets longer and longer. It is therefore argued on grounds of continuity

that before curarization the excitation time must have been within the 2:1 ratio. This conclusion, however, is not a necessary one, for it is at least conceivable that the action of curare on muscle might be like that of veratrine on nerve concerning which Lapique states [1926, p. 276] that the excitation time first diminishes, and then increases again to normal, curarization occurring in this latter phase. There is nothing in Lapique's experiments to exclude the possibility that the final excitation time of a curarized muscle is the same as the obscured initial value (or even shorter), for in no experiment so far as I know is any satisfactory evidence produced that the excitation time of muscle and nerve are normally identical.

But quite apart from the above criticism there are two others of much greater weight. Suppose it be granted that in Lapique's experiment the excitation time of muscle was initially near that of nerve, and finally very different, does it follow that the conduction block was due to this change? It will be shown that curarization may occur without any change of excitation time and again that the excitation time may change without curarization, that in fact this change when it occurs is merely incidental and in no way the basis of the conduction block.

Almost as soon as Lapique's theory appeared his experiments were repeated by Boehm [1910] in a very thorough investigation which failed to confirm Lapique in those very matters upon which his theory was based. Boehm excited muscles with condenser discharges of different rates and found that the constants of the Hoorweg equation altered when indirect excitation failed, but the new values were independent of the strength of curare or of the duration of its action. This work of Boehm therefore is consistent with the view that curare has no effect upon the excitation time of muscle or nerve but merely blocks the impulse at the myoneural junction. The observations are in complete contradiction with those upon which Lapique built his theory.

Three years before Boehm published his failure to confirm Lapique's experiments, however, some observations of Keith Lucas raised objections of a different kind to Lapique's theory of curarization. Lucas [1907-8*a*] stimulated the sartorius muscle through large fluid electrodes and obtained strength-duration curves with kinks in them which he considered to indicate the presence of three excitable substances. The slowest (α) was found in the nerve-free part of the muscle, was unaltered by curare and was identified with muscle, the fastest (β) was found only in the neural part of the muscle but not in a nerve trunk, it persisted after a dose of curare sufficient to abolish indirect excitation, and

was therefore identified with some intermediate substance between muscle and nerve. The γ excitability had the same excitation time as nerve and was abolished by a strength of curare which just removed indirect excitability and hence was identified with nerve.

It is clear that Lucas's results are completely contrary to the expression of Lapicque's theory, for neither are muscle and nerve normally isochronous, nor does curare have any effect upon the excitation of muscle.

The reason for the great divergence in the results of Lucas and Lapicque was brought to light in 1923 by the experiments of Jinnaka and Azuma, who used the nerve-free portion of the sartorius and stimulated with both large fluid, and small pore electrodes. They found that the excitation time of the muscle depended in a great measure upon the size of electrode used, but restricted their curare investigations to observations with pore electrodes, which they considered to produce a smaller "deformation" of current. In their experiments they avoided confusion between muscle and intramuscular nerve fibres by the use of their micro-electrodes, but their work failed to establish the effect of curare upon muscle excitation time, since they make no statement as to whether the doses of 0.03 and 0.05 p.c. employed actually removed indirect excitation or not. It is clear that they supposed that the smaller dose was sufficient, but attributed [with Mines, 1908] the effect upon excitation time to the presence of inorganic salts in the drug. They certainly found that Ringer's fluid containing 0.046 p.c. KCl caused the excitation time of the muscle to become considerably longer than did the strongest dose of curare, but no information is given as to whether this Ringer curarized or not. These experiments therefore pointed the way to an accurate analysis, but failed to supply the requisite controls, and it is not until this year that the method has been brought to full fruition by the work of Grundfest (see later).

Long before this, however, Watts [1925] extended the methods of Jinnaka and Azuma to give a contribution so definite that it has deserved a recognition far wider than it has received.

He stimulated muscles with electrodes of various sizes and investigated normal muscles, those after nerve degeneration and after paralysis by curare of various strengths. The experiments were not such as to allow accurate measurements both before and after treatment without shift of electrodes, but it is perfectly clear that weak but paralysing doses of curare do not appreciably lengthen the muscle excitation time (in fact they shortened it) and degeneration (about 40 days) had also practically no effect upon it.

In view of this historical summary therefore it is seen that the views of Lapique do not in the first place necessarily follow from his experiments, and the experimental results themselves are denied by the work of Boehm. Finally, the more penetrating analysis of Watts showed that normal isochronism was true or not, merely depending upon the size of electrodes employed (confirming Jinnaka and Azuma [1923] and Davis [1923]), but no matter what the size, there was no necessary change in the excitation time of muscle when paralysed by curare.

The case for Lapique's theory which is most complete and best known to physiologists is the account in his book [1926]. The weight of this argument, however, is very largely offset when it is recollected that Lucas's contrary experiments were discredited on the supposition (since retracted) that they were due to certain errors, that Jinnaka and Azuma are only discussed where their experiments do not bear upon the question of curarization, and that no mention whatever is made of Boehm or of Watts.

In view of the foregoing considerations it was apparent that whatever the actual truth of Lapique's views, the evidence put forward to support them needed considerable strengthening. In particular, experiments were needed which would distinguish clearly between the excitation of muscle and nerve in the fresh uncurarized condition, and which would give comparable results before and after treatment. Two methods presented themselves as the result of the observations of previous workers, either the use of large fluid electrodes in which case the muscle strength-duration curve is quite different from that of nerve, or the use of capillary pore electrodes where the stimulus may be localized to one particular excitable element. I adopted the first method and Grundfest the second, and it is satisfactory to find that in spite of the considerable difference in the techniques involved, our conclusions are identical.

The principle of the analysis by fluid electrodes is as follows. When muscles are stimulated in this way the strength-duration curve is often formed of two portions meeting at a kink as Lucas found [1907-8a]. If certain precautions are taken it can be shown that of these two portions the briefer (γ) is due to the intramuscular nerve twigs and the slower (α) to the normal muscle fibres themselves; I have never observed β . If this identification is accepted it is easy to investigate the effect of drugs, etc., upon these two curves and hence distinguish at once the action upon muscle from that upon nerve.

It was not possible, however, to assume at once the above identification of α and γ in view of the strong opposition of Lapique. This

opposition I claim to have met in a series of six papers in this *Journal*, the results of which are summarized in the last of them [Rushton, 1932c], and will not be repeated here.

*Curare and Block Electrodes*¹.

Upon the floor of a rectangular trough [Rushton, 1930b, Fig. 6] filled with Ringer's fluid a frog's sartorius was fixed by a pin through the pelvis and a thread from the tibial tendon to a light tension lever. The current was led through non-polarizable electrodes of Zn-ZnSO₄-Agar-Ringer, and was localized to the pelvic portion of the muscle by being concentrated under a Bakelite block 1 cm. broad and of thickness to fit the section of the trough, and so placed that its pelvic edge lay about 2 mm. from the pelvic bone. In this position when the cathode lay towards the pelvis the strength-duration curve was entirely α , but on reversing the current the curve was largely γ with a small α tail appearing at long durations below the γ rheobase (see Fig. 2). By this means a full α or a full γ curve could be observed by merely reversing the current.

A resistance of 10,000 ohms was placed in series with the tissue, and durations of current were controlled either by a Lucas long-range pendulum or by condenser discharges; the current strengths were varied by a high resistance potentiometer used in conjunction with an accurate voltmeter.

It is a familiar fact that the strength-duration curve of an excised sartorius changes considerably in the first few hours after excision, probably due to attaining a new equilibrium with the bathing fluid. In order to avoid complications due to these changes, muscles were not curarized until they had been left in Ringer's fluid for an hour or two and when the strength-duration curves appeared to have reached a stable condition. When at last equilibrium had been attained it was important not to disturb the salt balance in the addition of curare solution. To this end the curare (Merk) was made up concentrated in Ringer's fluid (1.0 p.c.) and 0.1 c.c. of this solution added to the 10 c.c. of fluid in equilibrium with the muscle. It was found that, after mixing, this concentration of the drug abolished excitation through the nerve in 10 min. In all these experiments the nerve to the sartorius was dissected out and placed (well away from the principal electrodes) on a subsidiary pair through which induction shocks could be passed. The moment of curarization could thus be obtained with certainty.

¹ These experiments were communicated to the American Physiological Society in March, 1930 [Rushton, 1930a].

Results.

The results of a typical experiment are shown in Fig. 1 plotted in logarithmic scales on both axes. The advantage of this manner of plotting (which has been discussed at the end of a former paper [Rushton, 1931]) is in the present case that the curve never runs too steeply to be easily followed, that the limits of error and exact positions of the experimental points may be clearly seen, that there is no confusion between the several curves appearing on a single figure, and that change of rheobase without

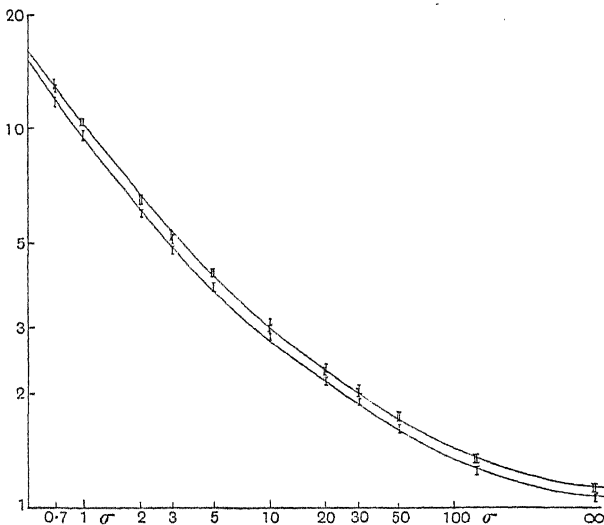


Fig. 1. Strength-duration curve of sartorius stimulated at pelvic end through block electrodes. Lower curve before curarization, upper curve after curarization and half an hour later. Abscissae log duration in σ , ordinates log strength of current.

change of excitation time is at once appreciated since in this case the whole curve moves bodily upwards or downwards vertically.

The procedure for the experiment of Fig. 1 was as follows:

The sartorius muscle with its nerve was dissected out in the morning and set up in the stimulating trough placed in a thermally insulated box cooled with ice to a stable temperature of 13.5°C .

- 3.30 p.m. The strength-duration curve was taken without opening the box (lower curve, Fig. 1).
- 4.01 p.m. The box was opened for a moment to administer a drop of concentrated curare, to make a solution of 0.02 p.c.
- 4.07 p.m. Slight response still from shock to nerve.
- 4.08 p.m. Curarization complete.

4.09-4.19 p.m. Strength-duration curve redetermined (upper curve right point of pair, Fig. 1).

4.40 p.m. Strength-duration curve repeated (upper curve left point of pair, Fig. 1).

Final temperature 12.5° C.

From the figure it is clear that the continued action of curare between 4.10 and 4.40 had not the slightest effect upon the threshold at any duration. It is interesting to note, however, that during this interval, a gastrocnemius muscle (from the same frog) which had also been placed in the trough, was gradually being curarized. This was accomplished at 4.39 p.m., taking $5\frac{1}{2}$ times as long as the sartorius.

With regard to the change in the curve before and after curare it is seen that the threshold is raised about 10 p.c. at all durations and that there is no change at all in the excitation time. This result is typical of all my experiments. In the earlier ones conditions were less well controlled and there was greater fluctuation, but this resulted as often in a slight shortening of excitation time as in a slight lengthening. The threshold changes were sometimes less than in Fig. 1, and sometimes more, and though never very great, always in the same direction. Even this, however, cannot be taken as a certain effect of curare, since a gradual rise of threshold commonly accompanies the ageing of the excised muscle apart from the administration of drugs.

Fatigue and block electrodes.

The tetanization of a nerve to a voluntary muscle soon brings about curarization, the mechanism of which is, according to Lapique, the same as in the case of paralysis by curare. Fig. 2, plotted upon uniform scales, gives the result of an experiment to find the effect of fatigue and subsequent curarization upon the α and γ curves of muscle.

The sartorius was set up as before and strength-duration curves were obtained (crosses) with the current towards the tibia (intermittent curve) and towards the pelvis (full curve). The nerve was then tetanized to complete failure of indirect excitation. The threshold was found to be the same whether the tetanizing current continued to be applied throughout, or whether it was stopped for a second while each strength-duration stimulus was sent in. It is seen (white circles) that curarization by fatigue has no effect at all upon the α curve obtained from the pelvic end of the muscle. On the other hand, the γ curve, initially prominent where the cathode lay towards the tibia, has completely vanished and in its place is seen an α curve which exactly joins the α portion initially appearing below the γ rheobase at durations greater than 15 σ .

It seemed of interest to observe the effect of curare upon the muscle

tetanized as above. According to the action claimed by Lapique it might be supposed that the excitation time of muscle would be increased by the drug, since he expressly states [1925] that there is no fixed upper limit to the muscle excitation time which might otherwise already have been attained by tetanization.

The effect of curare in these circumstances is seen in Fig. 2 (black circles), where it will be observed that there is no change whatsoever in the curve with cathode towards pelvis. With the reversed direction of the

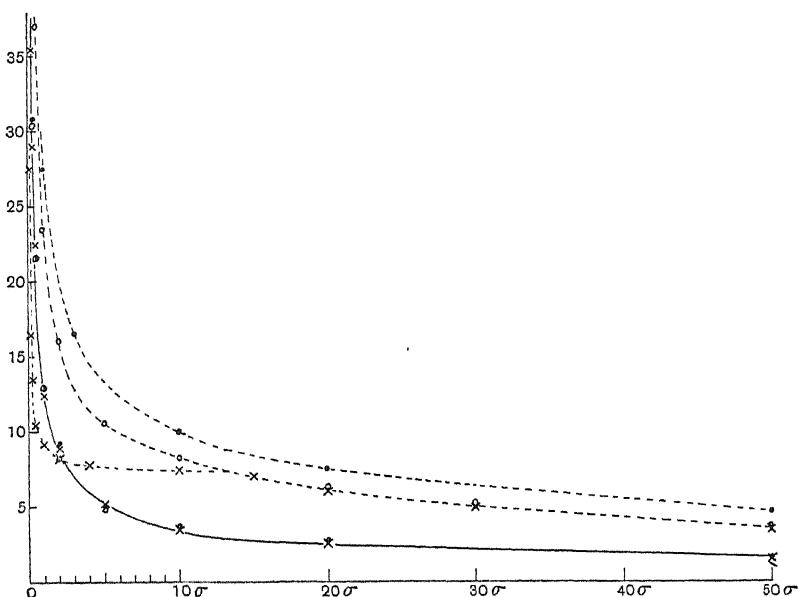


Fig. 2. Strength-duration curve of sartorius stimulated through block electrodes. Full curve cathode towards pelvis, intermittent curves, anode towards pelvis. Crosses before fatigue, white circles after fatigue, black circles after curare. Abscissae duration in σ , ordinates current strength.

current there is a small rise of threshold but no change in excitation time, hence not even in the region of nerve endings is any change in time relations observable.

Unfortunately this gives no information as to whether curare has any effect on the excitability of a muscle in the region of the nerve ending. If the drug made the muscle locally more irritable this would have appeared as a lowering of threshold, but if the muscle suffered the reverse change the only observed result would be the possible shifting of the most excitable fibre to one whose nerve did not enter in the neighbourhood of the applied cathode. This would be manifested as a slight rise in threshold for descending currents with

no change in threshold for ascending currents—which is in fact what is observed, but the evidence is far too slender to infer that the results were necessarily produced in the above manner.

These experiments therefore confirm the results of Lucas and Watts with large electrodes, for not only is there no change of excitation time due to curarization by fatigue or curare, but also there is practically no threshold change at all.

I next sought to discover which of the differences between Lapicque's technique and my own were responsible for the differences in the apparent action of curare.

Condensers.

Lapicque has usually employed condensers on account of their convenience and accuracy at short durations, but he claims that the results with curare are equally found when brief constant currents are employed. I also confirmed that the condenser method is not responsible for the divergent results, for with their aid I still found curare to have no effect upon the muscle threshold.

Gastrocnemius.

Lapicque's observations on the effect of curare were made upon the gastrocnemius: the sartorius he claimed to be anomalous. In my experiments with undrugged muscles [1931] it had appeared that the sartorius, gastrocnemius and a dozen other muscles all behaved in much the same way with regard to strength-duration curves, except that it was easier in the sartorius to avoid stimulating the intramuscular nerve twigs (which might make it more difficult consistently to obtain isochronism between muscle and nerve). However, it seemed worth while to repeat the curare experiments upon the gastrocnemius, using fluid electrodes and the same technique as before. The result of one experiment is shown in Fig. 3 (plotted in logarithmic scales), where the procedure was as follows:

- Temp. 12° C. 4.0 p.m. Normal strength-duration curve (intermittent line).
4.15 p.m. 30 mg. curare added to bathing fluid (10 c.c.).
4.47 p.m. Curarization complete: strength-duration curve (crosses).
5.48 p.m. Strength-duration curve (white circles).
9.48 p.m. Strength-duration curve (black circles).
Temp. 12° C.

It is seen at once that the results here are the same as with the sartorius. The initial curve shows a prominent γ portion because of the difficulty of finding a nerve-free part of this muscle, but there is quite

sufficient of the muscle curve (durations greater than 7σ) to make it clear that after curarization (crosses) the threshold and excitation time are unaltered. As for the continued action of the drug prolonging more and more the muscle excitation time, so far from this being the case, after 5 hours' immersion there is no change that cannot be put down to alteration of ionic equilibrium (much slower than with the sartorius), and in any case the excitation time is not prolonged, but diminished.

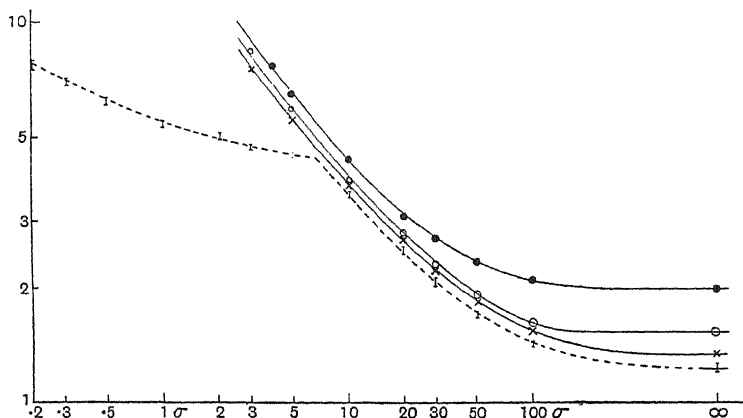


Fig. 3. Strength-duration curve of gastrocnemius stimulated through block electrodes. Intermittent curve before curarization, crosses, white circles, black circles, immediately after curarization, 1 hour, and 5 hours after curarization respectively. Abscissae log duration in σ , ordinates log strength of current.

Nature of the curare.

The curare which is placed upon the market is by no means a pure chemical, and it seemed quite possible that among the constituents there were two quite distinct substances one of which paralysed and the other independently altered the excitation time of muscle. According to this idea the Sorbonne curare had a much larger quantity of the second constituent than either the Cambridge curare, the sample which I obtained from Merck, or the specially purified product used by Boehm. I was enabled to investigate this possibility through the kindness of Prof. Lapicque, who sent me two samples of the drug one of which was from the supply which he had used for most of his experiments (curare "Type").

This curare was very effective in removing indirect excitation, but otherwise behaved exactly like the sample with which I had already experimented. In fact the actual examples quoted in Figs. 1 and 3 were

performed with Lapique's "curare type." It is clear, therefore, that whatever differences may lie in different samples of curare, the increased excitation time of muscle observed by Lapique cannot be attributed to this cause. This has been further confirmed by Lapique, who finds [1930] that with large electrodes the excitation time is if anything diminished by curare but with stigmatic electrodes it is greatly prolonged.

Nature of electrodes.

The experiments described in this paper so far have all been made with large fluid electrodes, since by that means nerve and muscle may easily be distinguished and because the nature of the contact between muscle and electrodes is not altered by the application of the drug. In view of Lapique's observation, however, it appears that the investigation should be repeated using small electrodes. It is here that the recent experiments of Grundfest [1932] are of the first importance. Grundfest has investigated the retro-lingual membrane of the frog by means of capillary electrodes. The experiment is easy to appreciate and the results are clear.

First the capillary cathode is placed over a small nerve twig which is stimulated by a condenser discharge. A group of muscle fibres respond and of these one is chosen for observation. The voltage-capacity curve of the nerve is then taken using this muscle fibre as index. Next the cathode is applied directly to this muscle fibre and the voltage-capacity curve obtained, when it is thus directly excited. The cathode is replaced on the nerve, curare is added, and the efficacy of curarization ascertained by failure of response to indirect stimulation. Finally the cathode is again placed on the muscle as before and the voltage-capacity curve re-determined.

The results are quite definite. Initially the excitation time for nerve is about ten times as brief as for muscle. Curare has no effect whatever upon the excitability of muscle, either in threshold or excitation time. These results are therefore in complete accord with those where large electrodes are used and directly contradict Lapique's claim as to the difference in the two cases.

In order to complete this type of investigation it seemed worth while to repeat these observations with electrodes larger than Grundfest's and more akin to those of Lapique. First I attempted to use an Ag-AgCl wire applied vertically to the surface of the pelvic region of the sartorius but not pressing. I found it impossible to get reproducible results by this method even in the absence of drugs, and this at the time

I attributed to the polarizability of the stigmatic electrode (even though the stimulus was reversed between each observation). A more probable explanation, however, is the toxic effect of this form of electrode when applied directly to muscle [Rushton, 1932*b*]. More repeatable results were obtained by using a relatively large capillary, of internal diameter 0.26 mm., applied to the pelvic end of the sartorius, the large indifferent anode being placed over the point of nerve entry. Whether the muscle was covered with Ringer or whether it was in air when strength-duration curves were taken, made no difference to the result that curare in a dose sufficient to abolish indirect excitation had no effect upon the muscle excitation time.

The following two examples are typical.

Exp. 1. Preparation set up at 12.0 m. as described, with 12,000 ohms equivalent series resistance in condenser circuit.

12.0 m. Rheobase 7.5 volts. Excitation time 0.046–0.044 μ F.

2.0 p.m. Rheobase 15.0 volts. Excitation time 0.084–0.080 μ F.

Langley's curare added, abolishing the indirect excitability in 3 min. The excitation time was now 0.086–0.083 μ F.

Exp. 2. Preparation set up at 10.5 a.m., resistance in condenser circuit 10,000 ohms.

10.40 a.m. Rheobase, 4.0–3.8 volts. Excitation time, 0.084–0.080 μ F.

10.55 a.m. Langley's curare added, abolishing indirect excitability between 11.0 and 11.1 a.m.

11.5 a.m. Rheobase, 4.0–3.8 volts. Excitation time, 0.094–0.090 μ F.

11.45 a.m. Rheobase, 5.5–5.2 volts. Excitation time, 0.090–0.086 μ F.

From Exp. 1 it is seen that there is a large change in the excitation time of the excised muscle in the first hour or so after excision and setting up in Ringer's fluid due to the new ionic balance. This change is nearly in the ratio 1:2, yet no sign of conduction block was ever seen on indirect excitation. Obviously if curare were administered in this interval the impression might arise that it was the drug which was prolonging the excitation time. This is clearly incorrect, for not only does this prolongation occur without the application of curare, but also the subsequent administration of the drug produces no change in excitation time at all. This is confirmed by Exp. 2, where it is seen moreover that the prolonged action of the drug produces no effect. These experiments clearly exhibit a fair degree of reliability and stability of threshold, and the results are in complete accord with Grundfest and the earlier conclusions of this paper.

Strength of curare.

In all my experiments a weak solution of curare was used, 0.01–0.02 p.c., which curarizes the sartorius in 5–10 min. but takes nearly an hour with the gastrocnemius. It is certain that in some, and possibly in most

of Lapique's experiments a much stronger solution was used, and it is easily conceivable that such a solution might prolong the muscle excitation time. I have not investigated this point, since it is clear that if a weak but curarizing dose does not alter excitability, the chronological theory falls, no matter what results arise with stronger doses. Moreover, since Mines showed [1908] that the excitability change produced by the drug was the same as that produced by the ash of an equal quantity of drug (hence due to its inorganic content) it is not unlikely that this matter is merely one of salt balance and has nothing to do with curarization.

We may summarize the analysis of Lapique's first argument as follows:

(i) In all the experiments known to me where the direct excitation of muscle has been clearly distinguished from the excitation of intramuscular nerve twigs, nerves have always been found to have a shorter excitation time than muscle measured with the same electrodes whatever the size.

(ii) Curare has no action upon the excitation time of muscle as observed by Boehm or myself using the same size of electrodes as Lapique, or by Grundfest using smaller ones, or by Lucas, Watts or myself using larger ones.

Thus with regard to Lapique's first argument to support his theory of curarization, we have seen that in the first place it is inconclusive, admitting equally of the conclusion of initial heterochronism; in the second place, even granting his observations, there is no reason to suppose that the change of excitation time which he obtains is in any way related to the curarizing action of drugs; and in the third place his observations are denied by all investigators whose work has clearly distinguished between the direct excitation of muscle and the stimulation of nerve twigs.

We pass to Lapique's second argument.

LAPICQUE'S SECOND ARGUMENT.

The second of the three arguments employed by Lapique to justify his chronological theory of excitation relates to an antagonism which he finds between the drugs strychnine and veratrine with regard to their curarizing actions. The argument is briefly as follows. Strychnine has no effect upon the excitation time of muscle, but that of nerve is shortened and at the moment of abolition of indirect excitability there is a heterochronism in the ratio 1:2. Curarization is supposed to be the result of this heterochronism as in the case of curare. Veratrine on the other hand

curarizes at a moment when the nerve excitation time is unaltered, but that of the muscle shortened. Now if in fact curarization is due to divergence in excitation time, it is clear that after curarization by veratrine indirect excitation should be restored by strychnine or *vice versa*. This in fact is claimed to be the case, thereby constituting an interesting confirmation of the theory.

But evidence which is amply adequate for the confirmation of a theory may be quite insufficient for its initial establishment and it is very doubtful whether the strychnine-veratrine antagonism would ever have led to Lapique's theory if he had not already put it forward upon other grounds. Those grounds we have already considered in the previous section of the present paper and have seen that they are very inadequate, for not only is the theory not supported, but it is definitely rendered untenable by the observation that paralysis by curare may (and in my experience always does) occur without any change in the excitation time of muscle or nerve. The most favourable observations of strychnine-veratrine antagonism, therefore, could not justify Lapique's theory in the face of the foregoing evidence. Yet since a systematic correlation between drug antagonism and the opposite effects of the individual drugs upon the excitation time of nerve and muscle might not be without value, I have attempted to confirm Lapique's observations upon this matter. It will be unnecessary, however, to describe the majority of the various experiments performed, since that which is at once the simplest and most crucial shows that strychnine and veratrine do *not* antagonize each other.

In this experiment I followed Lapique in that I used the frog's sciatic gastrocnemius preparation, and employed the solutions he mentions [1926] namely strychnine HCl 0.3 p.c., veratrine HCl 0.1 p.c. In the first variety of this experiment the central end of the nerve was screened from the drug and electrodes were applied to the screened portion. By this arrangement the excitability of the nerve under the electrodes was unaffected by drugs, and the possibility of a conduction block occurring at the place where the nerve passed from the screened to the unscreened region was excluded by controls which showed that conduction block was never produced in this way. By means of a simple piece of mechanism an induction shock was applied to the nerve automatically every minute, and the resulting contraction recorded on a very slowly moving drum. The results of two typical experiments are shown in Fig. 4, where the tracing is to be read from right to left. Owing to an imperfection in the mechanical contact, occasionally a double shock was administered pro-

ducing summation, but apart from this, the records are very regular. In the upper tracing there is a uniform height when in Ringer's fluid and immediate summation and contracture when 0.1 p.c. veratrine was added followed by a rapid decline with complete curarization in half an hour. Now the veratrine was removed and replaced by 0.3 p.c. strychnine, but though the record was continued for several hours beyond what is shown in the figure, no sign of a twitch was observed. These strengths of drug were as recommended by Lapicque, and yet the strychnine seems still too weak to neutralize the veratrine. The lower tracing therefore shows the repetition of the experiment on the other leg of the same frog where

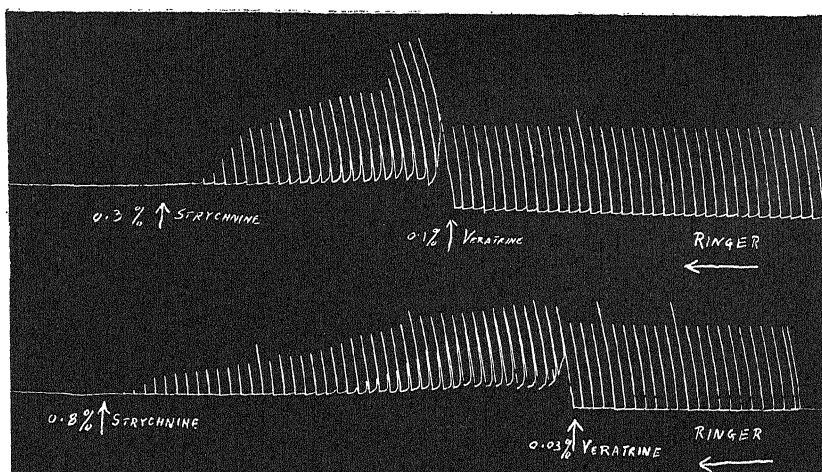


Fig. 4. The effect of veratrine and subsequent application of strychnine upon nerve-muscle conduction (see text).

the strychnine-veratrine ratio was increased nine times. As would be expected paralysis is slower, but here too there is no restitution of conduction. There was no single exception to this result; never for the space of a minute did strychnine restore the conduction abolished by veratrine, although the strychnine solution was so strong that one drop in the lymph sac of a spinal frog produced an intense spasm within 5 min.

Prof. A. V. Hill was good enough to point out an important possibility which must be considered, however, in connection with this analysis. It is known that in a veratrine contracture a very great deal of energy is expended, hence in the repeated contractures which were elicited in the foregoing experiment a considerable degree of fatigue was

doubtless attained. The question arises as to whether curarization was due to fatigue and not directly to veratrine at all, for if this were the case no resuscitation by strychnine would be expected.

To investigate this possibility the experiment was modified slightly as follows. Two preparations, *A* and *B*, from the same frog were set up side by side in the way described above, and after initially ascertaining that both were irritable and in good condition shocks were applied to *A* only. It is obvious that if curarization of *A* is due to fatigue as suggested, *B* will always be excitable at the moment when *A* fails. If on the other hand the fatigue has nothing to do with the question *B* will be paralysed before *A* as often as not. This latter was actually the case, for in slightly more than half the experiments *B* had already been curarized by the time that *A* failed. A typical example is as follows:

4.20 p.m. 0.1 p.c. veratrine HCl solution added.

Every 2 min. a shock is sent into preparation *A*; *B* is rested.

4.44 p.m. *A* still contracts.

4.46 p.m. *A* curarized; *B* curarized, though now excited for first time.

4.48 p.m. Veratrine poured away and 0.3 p.c. strychnine HCl added.

4.50–5.50 p.m. Shocks applied to both preparations every minute for next hour without ever evoking a response.

From results such as those it is clear that curarization of *B* cannot be due to fatigue, since there was no fatigue and hence in these cases at least Lapique's drug neutralization does not occur. Since, moreover, the evidence leads us to suppose that even when fatigue is present it has no effect on the veratrine curarization, we may also accept the experiment of Fig. 4 as trustworthy.

But though the foregoing experiments have shown that strychnine will not antagonize the veratrine curarization when used in Lapique's concentrations or when the strychnine-veratrine ratio is considerably increased, are we to conclude that Lapique's theory is inoperative? Is not the alternative equally plausible, that the strychnine used was at strongest too weak to reduce the nerve excitation time to the diminished value which the applied veratrine has produced in the muscle? A slight modification of the foregoing experiments gives definite evidence on this matter. It is obvious that if strychnine neutralizes the action of veratrine as Lapique supposes, by shortening the excitation time of the nerve to the diminished value assumed by the drugged muscle, then, equally, veratrine will neutralize the curarization with strychnine by bringing about the same final condition. This order of performing the experiment avoids completely the complications of the veratrine contracture, since

veratrine is not applied till after curarization by strychnine has been brought about, and in no case was any further movement of the muscle observed.

A typical experiment is as follows:

- 5.5 p.m. Preparation immersed in 0.3 p.c. strychnine HCl.
- 5.30 p.m. Still excitable.
- 5.35 p.m. Curarized.
- 5.37 p.m. Strychnine replaced by 0.1 p.c. veratrine HCl.

The preparation was stimulated for the next half hour at first every $\frac{1}{2}$ min., later at 1 min. and 2 min. intervals, but no excitation ever returned.

It is seen that this case confirms all the foregoing. Curarization by strychnine could never be abolished by the replacement of this drug by veratrine in the strengths used by Lapique. These results, moreover, cannot be explained upon the grounds of unsuitable drug concentrations, since in this case the *veratrine* would have to be too weak to reduce the muscle-excitation time to the diminished value produced in the nerve by the strychnine, which is precisely contrary to the conclusion of the previous experiment. In short, there is never restoration of conduction, no matter which drug is applied first, and hence this failure cannot be due to the first drug being always the stronger.

In all these experiments there can be no question of large or small electrodes, true or false chronaxies. We have here only to consider whether an impulse can or cannot be conducted, whether a muscle twitches or not. If Lapique's theory were true, the antagonism would have to occur every time—a few exceptions would render it untenable. But in my experience the exceptions so far from being few are universal. In no single case have I ever seen this antagonism which Lapique's theory would inevitably demand.

With regard to Lapique's second argument therefore, we may conclude that at best the strychnine-veratrine antagonism could only confirm by exceptionless regularity a theory established upon other grounds. But these other grounds are wanting, and the drug antagonism is in my experience never obtained.

We pass to Lapique's third argument.

LAPICQUE'S THIRD ARGUMENT.

Lapique's third argument in favour of his chronological theory of curarization is comparatively recent [1925] and makes use of the fact investigated by von Kries [1884] and Keith Lucas [1907-86] and

himself [1908] that if a current increases from zero somewhat slowly it may never excite a nerve but may still be capable of eliciting a propagated contraction from muscle. As a means of demonstrating the presence of muscle in the nerve-muscle complex this method is a very elegant one, but it gives us no information about the excitation time of the muscle unless we assume with Lapique that the minimum rate of current increase which will excite is inversely proportional to the excitation time, whatever the tissue and whatever the condition of the tissue. This is a very bold assumption, and some substantiation would surely not have been out of place, especially since the only experiments I know which test the matter directly [Lucas, 1908] show quite contrary results. The only justification which is given, however, is the following mathematical generalization. If the stimulating current is any function of time $f(t)$, and if τ is the excitation time of the tissue then $f\left(\frac{t}{\tau}\right)$ is an expression independent of the tissue investigated or of the condition of the tissue. This generalization is far more sweeping than the particular case of it considered by Lapique in his third argument, and consequently it needs the more extended substantiation. The proof of such a statement must be a most laborious work, involving as it does stimuli of diverse kinds, applied to various tissues, in all sorts of conditions. Whether Lapique's generalization has in fact ever been supported by such a series of investigations or not, I do not know. I am only aware of one particular case which has been studied with the necessary care—that of the strength-duration curve—and here $f\left(\frac{t}{\tau}\right)$ is not independent of the tissue, being what Lapique calls “canonical” in slowly reacting tissues but “uncanonical” in frogs' nerves [Rushton, 1932*a*].

As a result of this analytical survey therefore we may conclude that Lapique's generalization is at best unproven, and probably incorrect, and that the particular case of it which he uses for his third argument lies in the same category. Thus even if we accept his experimental conclusions we cannot deduce anything from them concerning the excitation time of the tissues involved nor learn anything concerning his chronological theory of excitation. The conclusions in question, however, were not experimentally confirmed.

Lapique's experiment [1925] was as follows. The nerve-muscle complex is excited directly through a stigmatic cathode by the apparatus shown in Fig. 5. If first the capacity C is made zero, then when the key K is closed a constant current will flow through the muscle, as shown in Fig. 6*A*. The threshold is determined, and is in fact the rheobase. Now a

small capacity is given to C , and when K is closed the current through the tissue increases as shown in Fig. 6 *B*. The threshold for this current will be greater than the rheobase. If now the capacity of C is still further increased the current will rise still more slowly on closing K (Fig. 6 *C*) and the threshold will be still higher.

The consideration of the curve relating the threshold with the capacity of C both before and after curare led Lapicque to two conclusions:

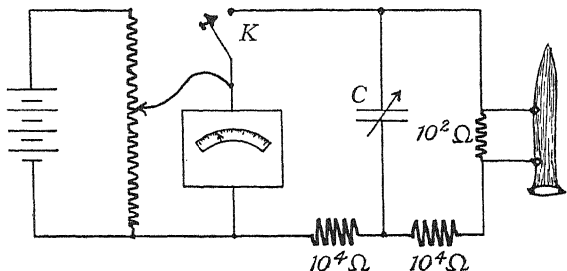


Fig. 5. Lapicque's circuit for excitation with slowly increasing currents.

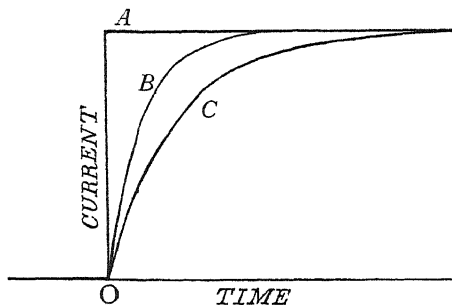


Fig. 6. Time course of current through muscle on closing K , Fig. 5.

(a) Since before curare the curve was the same as for nerve he concludes that nerve and muscle are normally isochronous.

(b) Since after curare the threshold for abrupt currents is raised while for slowly rising currents it is lowered he concludes that curare prolongs the excitation time of muscle.

But even if we neglect the foregoing criticism neither of these conclusions can be accepted without further investigation. For (a) may be equally interpreted as due to the intramuscular nerves being excited, in which case naturally the curve would be the same as in the case of the nerve trunk, and we learn nothing about the muscle except that its threshold is higher than that of nerve—the rheobase perhaps three times

as great. As Lapique mentions no precautions to exclude nerve twigs this must be regarded as a very likely explanation. But in this case (*b*) may merely signify that after curare has removed the nerves, the remaining curve is the normal muscle curve, unaffected by the drug. Against this Lapique argues that were it the case the lower threshold at large capacities would have appeared in the initial curve (the lowest threshold in every case obviously being taken). The validity of this depends upon the stability of the threshold measurements. Clearly if during the process of curarization there was a change in the exact point of stimulation, in the degree of moisture of the surface, or of the nature of the contact, etc., these physical conditions might produce considerable threshold changes. Lapique himself, in urging the relative stability of the chronaxie as a measure, has emphasized the variability of the rheobase—at least when obtained through stigmatic electrodes in air. Can we rely upon this rheobase before and after curarization to be so stable that some lowering due to physical causes is out of the question? It seemed worth while to repeat Lapique's experiments bearing in mind these criticisms.

To this end I followed Lapique's methods as closely as was compatible with certain controls. The electric circuit was exactly as described [Lapique, 1925]. It was found impossible to get repeatable results using Ag-AgCl electrodes or with muscle completely in air, hence a glass capillary of 0.26 mm. bore was used as cathode and applied to the surface of the muscle just emerging from a Ringer bath. The muscle used was the frog's sartorius with nerve dissected out; the cathode was placed near the pelvic extremity; the anode, which was a tube of 7 mm. diameter, was fixed over the place of nerve entry. From a former paper [Rushton, 1932*b*] it is clear that nerves are less likely to be excited in the nerve-muscle complex if the anode is placed over the nerve entry than in any other position.

By tilting the trough it was possible to immerse the muscle or cause the fluid to drain back again without disturbing the electrodes, and if a minute or so was allowed for drainage the results were found to be very fairly reproducible as the following example shows. The threshold for each case lies between the two figures quoted; their difference shows the experimental limits.

The preparation was set up at 4.10 p.m., and it is clear that over the first hour there is a considerable variation due to change of equilibrium. Obviously anyone curarizing in this period might be led to suppose that it was the curare which was raising thresholds for small capacities more than for large (as Lapique claims). It is moreover interesting to note

that although the change in the first two columns are almost as great as those which Lapique finds with curare, yet no sign of curarization was revealed by exciting through the nerve. On the other hand, adding a drop of concentrated curare to the bathing fluid and tilting the trough caused curarization in 8.5-9 min., without producing any change other than a slight rise of threshold for all capacities. The figures show beyond doubt that neither at the moment of curarization nor for 40 min. afterwards was there any significant change of threshold due to curare, which only confirms the earlier experiments of this paper. This experiment, which is typical of all which I have performed, therefore, leads to the following conclusions.

Capacity	Thresholds.					
	Before curarization		After curarization			
	4.30 p.m.	5.0 p.m.	5.20 p.m.	5.25 p.m.	6.0 p.m.	
0.0 μ F	9.0- 8.5	13.5-13.0	14.0-13.5	14.5-14.0	13.5-13.0	
0.3 μ F	9.5- 9.0	13.5-13.0	14.5-14.0	15.0-14.5	14.0-13.5	
1.0 μ F	11.5-11.0	14.0-13.5	15.0-14.5	15.5-15.0	14.5-14.0	
3.0 μ F	14.5-14.0	15.5-15.0	17.0-16.5	17.0-16.5	16.5-16.0	
10.0 μ F	22.0-21.0	20.0-19.0	22.0-21.0	23.0-22.0	22.0-21.0	
0.0 μ F	9.0- 8.5	13.3-12.8	14.5-14.0	14.0-13.5	14.0-13.5	

Curare added at 5.10 p.m., indirect excitation abolished between 5.18½ and 5.19 p.m.

When first set up the muscle is unstable and no observations are significant for an hour or so (confirming former investigators). When the capacity-threshold relation is taken this does not coincide with Lapique's results with nerve, but more closely with his results with muscle after nerves had been removed by curare. All thresholds after curare are (within experimental limits) exactly the same as before curare.

These results are quite irreconcilable with Lapique's conclusions, but they are easily reconcilable with his observations which apparently should be interpreted as suggested earlier, namely, that initially he was stimulating nerve twigs, and when these had been removed by curare he was stimulating the normal muscle.

To summarize. Lapique's third argument deals with the excitability of tissues to slowly rising currents. No relation was established, however, between this form of excitation and the excitation time, hence measurements upon the one could not be used to establish a curarization theory involving the other, however sound the experimental conclusions. These conclusions, however, lay themselves open to an entirely different interpretation, which the present experiments confirm, namely that curare has no effect upon the excitability of muscle.

DISCUSSION.

In the former parts of this paper we have considered the evidence which Lapique has brought forward in support of his chronological theory, and have observed that each of his arguments is doubly inconclusive. In the first place the deductions which are drawn from the experiments are not inevitable, and explanations other than Lapique's would fit equally well—on this account the chronological theory is possible but unproven. In the second place the very experiments are not confirmed when various precautions are taken as already described—on this account the chronological theory is not merely unsupported but untenable.

Whatever may be the mode of action of curare, it certainly is not to alter the excitation time of the muscle fibre at least over the greater part of this structure. Whatever may cause conduction block it is not the production of heterochronism, for no observable change in excitability exists.

If then chronaxie does not play the important physiological rôle that has been attributed to it, if it is not the pass-sign of nerve-muscle conduction, what, finally is the value of this measure? This greatly depends upon the meaning we assign to "chronaxie." If we mean a measure satisfying all Lapique's recent restrictions—obtained only from a curve which is canonical, with electrodes not large enough to give the " α complication" nor small enough to give the "Grundfest heterochronism"—then clearly the utility of chronaxie will be limited. If a more general meaning is assigned, the measure has still great value in physiology, but to avoid confusion it is essential to use a name distinct from "Chronaxie," which according to Lapique may no longer be employed in this sense. Lucas's "Excitation time" exactly meets our requirements. Excitation time therefore is the measure characterizing the rate of development of the excitatory process in given conditions.

It is important to note that in general the term "conditions" will comprise two factors, the tissue (involving its nature, temperature, salt balance, etc.) and the physical conditions of stimulation (involving the distribution of current over the surface of the tissue). In certain particular cases the second factor will make little difference to the result; for instance, a sciatic nerve gives an excitation time relatively independent of the nature of the electrodes, possibly because the nodes of Ranvier are the effective electrodes in this case and because all the nodes are very similar. But familiarity with nerves has given rise to the erroneous im-

pression that independence of the conditions of stimulation is the rule instead of the exception (even though with nerves there is appreciable dependence), and hence it must be emphasized that unless the contrary has been established it should always be assumed that excitation time will vary with the nature of the electrodes.

The reason for this variation has not yet been established with certainty, but since it follows as a logical consequence from the commonly accepted theory of excitation [Rushton, 1932*c*] this explanation seems obvious enough and might have been accepted without further comment had not Lapicque put forward an alternative [1932]. A theoretical discussion of the excitation process obviously lies outside the scope of the present paper, but since Lapicque's alternative assumes that excitation time in general does not measure the duration of the process of excitation but the sum of this and another large extraneous factor, it is important to consider this question when dealing with the measurement and significance of the excitation time.

It is generally agreed that the passage of the stimulating current through the tissue polarizes the membranes through which it passes and thus produces a counter E.M.F. which tends to disperse the polarization. For this reason the polarization is developed not only under the electrodes but gradually further and further away as has long been known from investigations upon electrotonus. Now from Nernst onwards it has been commonly supposed that excitation is the direct result of the establishment of a certain critical ionic concentration (at least if this be attained within a very brief interval), and so it is important in considering Lapicque's suggestion to know whether he accepts this view or not. In the absence of any explicit statement or discussion upon this head we should hardly have expected a new but tacit departure, yet from his paper not only is the above polarization treated as a phenomenon distinct from the basis of excitation, but apparently excitation itself cannot begin to develop until the polarization has been arrested. This polarization moreover has some surprising properties. When it spreads down the length of a fibre, for example, it is stopped by the wall of a glass tube placed outside the connective tissue over the surface of the muscle, and this stoppage apparently arrests the increasing polarization at a distant spot under the cathode.

However, apart from this doubtful physics Lapicque and I are in complete agreement in considering that excitation time measures the duration of the polarization process (at least when large fluid electrodes are concerned) and thus it measures that process which is generally

accepted as underlying excitation. The only divergence is that for some reason unspecified Lapique appears to have tacitly rejected the classical connection between polarization and excitation, treating the two as separate and somewhat opposing entities, but explaining his position no further. There is obviously here no necessity for abandoning the polarization theory of excitation, and since there is general agreement that excitation time characterizes the rate of polarization, it consequently measures the rate of development of the excitatory process.

The arguments urged on theoretical grounds against large fluid electrodes fall with the theory upon which they were based, and electrodes of the block type not only are admissible but are probably the most satisfactory of any for work on the excitability of excised tissues. Among their advantages are the following:

(a) Stability of threshold resulting from the uniformity of ionic environment, the absence of desiccation, the constancy of contact, and the relative unimportance of small movements.

(b) Ease of investigating with stable threshold the effect of drugs, etc.

(c) Obvious distinction between excitation of muscles and nerve twigs (α and γ curves).

(d) Knowledge of the potential applied to every part of the surface of the tissue. This knowledge is a first essential for any theoretical treatment concerning the current entering the tissue, for without it the proportion of the applied current which enters cannot be known.

CONCLUSIONS.

Two and a half decades have passed since Lapique first put forward his chronological theory of curarization, and during this time a very large number of publications have appeared based upon these ideas which have become widely accepted.

His position was definitely established when in 1926 he published his well-known book which by its exceptional interest and lucidity has deservedly won a place in every physiological library. For the main thesis of the book we can have nothing but admiration. It represents Lapique's chief contribution to physiology, namely the demonstration that tissues of apparently the most diverse kinds differ essentially in only one respect—the scale of time in which they act. Extend or diminish this scale and the gross differences vanish. But the exposition of his theory of curarization could not be so unreservedly accepted. A chapter

of 40 pages deals in detail with this theory, and yet, as we have seen, the evidence is inconclusive, the experimental discussion has some important omissions, and the treatment of Lucas's results is not convincing. It was obvious that further work was still needed either definitely to establish or definitely to disprove this important theory which was yearly being more widely accepted as the basis of new departures.

During the past four years I have attempted to do this, and the present paper completes my investigation. The principal results which have emerged are the following:

(a) When any striated muscle is excited through fluid electrodes two kinds of strength-duration curves may be found either single or combined in proportions which may be altered at will. One of these (γ) is identical with nerve, the other (α) very much slower.

(b) A variety of experiments shows that α is due to the excitability of one element and γ of another.

(c) Various kinds of abnormalities were excluded, hence this dual excitability must be considered normal.

(d) A detailed analysis proves α to be muscle, and γ invariably the intramuscular nerve twigs.

(e) By this technique curare is found to have no action upon muscle excitability, for the α curve is unchanged, though the γ vanishes.

(f) Curare is also without effect upon the muscle strength-duration curve elicited by a capillary cathode applied to the nerve-free portion of the sartorius.

(g) Curare is also without effect upon the excitability of the muscle to slowly rising currents.

(h) Curarization by strychnine was never abolished by veratrine, nor *vice versa*.

In fact in these experiments where the direct excitation of muscle has been clearly distinguished from the excitation of the intramuscular nerve twigs, the latter have invariably been found to have a shorter excitation time than muscles stimulated through the same electrodes whatever their nature.

Neither curare nor fatigue has been found to have any effect whatever upon the muscle threshold no matter what the nature or duration of the stimulus.

These observations, therefore, do not substantiate Lapicque's theory of curarization, but on the contrary they render that theory untenable.

SUMMARY.

1. The paper attempts a critical and experimental study of Lapique's theory of curarization.

2. When precautions are taken to avoid stimulating the intramuscular nerves in the uncurarized preparation by (a) using "block" electrodes and observing the α curve, or (b) using stigmatic electrodes applied to the nerve-free part of the muscle, curare is found to paralyse without affecting the strength-duration curve of the muscle in any way.

3. This is in complete agreement with the recent observations of Grundfest and with many former workers.

4. Lapique's claim as to the antagonism of strychnine and veratrine was not confirmed. In no single case was curarization by one drug removed by the application of the other.

5. Lapique's most recent argument in favour of his curarization theory relates to excitation by slowly increasing currents. This argument is criticized. His experiments lie open to the interpretation that curare has no effect upon muscle excitability; initially not muscle but the intramuscular nerve twigs are excited, and only after curarization is the muscle directly stimulated. That Lapique's observed excitability change upon curarization is due to this transfer from nerve to muscle, is confirmed by the present experiments into which initial nerve excitation does not enter.

6. Thus there is positive evidence against Lapique's theory of curarization, and no unequivocal evidence in its favour.

This paper completes the account of the experiments I made in the University of Pennsylvania, and I should like to express here my sincere appreciation of the welcome and assistance which I received in Philadelphia, and especially to thank Prof. Bronk and Mr A. J. Rawson.

It is also a pleasure to own my indebtedness to the Government Grants Committee of the Royal Society.

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THE EFFECT OF INSULIN ON THE RESPIRATORY
QUOTIENT, OXYGEN CONSUMPTION, SUGAR
UTILIZATION, AND GLYCOGEN SYNTHESIS
IN THE NORMAL MAMMALIAN HEART
IN HYPER- AND HYPOGLYCÆMIA.

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WHILE attention has been largely focused on the action of insulin on the mammalian organism as a whole under conditions of hyperglycæmia or maintained blood-sugar levels, comparatively little attention has been directed to the action of insulin under hypoglycæmia conditions. The many problems of carbohydrate metabolism which have been opened up by the discovery of insulin still demand that the functions of the organs of the mammalian body be individually investigated as a logical step towards the elucidation of the action of this hormone upon the body considered as a whole.

The results to be presented and discussed here are the outcome of previous work upon the action of insulin on the normal and diabetic heart. In certain respects the diabetic heart does not apparently behave as does skeletal muscle and whether or not the normal heart should be expected to conform in all functional respects to skeletal muscle is still an open question. That one of the best means by which to determine the reaction of skeletal muscle to insulin is the use of the eviscerated spinal preparation as suggested by Burn and Dale [1924] and others few will deny; but that it is a good standard by which to compare the results of similar investigations upon cardiac muscle is a moot point. And further, it is essential that, in assessing the value of results obtained on cardiac muscle, one should not be obsessed by the findings recorded for skeletal muscle. Such investigation demands that the heart be allowed to function in as normal a condition as possible: to remove the heart from the body of an animal, wash it in saline or Locke-Ringer solution, remove adherent tissue from it, attach it to a perfusion apparatus and then perfuse it with Locke-Ringer solution or diluted blood is certainly not the best means of securing figures indicative of the normal reactions of that organ. Despite its disabilities the heart-lung preparation of Starling

is still of value; the preparation can be completed in 15 minutes, the heart suffers no handling, is supplied with its own blood, and the lungs assure an efficient oxygenation of the blood, and where the gaseous metabolism of the heart-lung preparation must be determined both the circulatory system and the respiratory system can be effectively closed.

METHODS.

To arrive at an accurate idea as to the gaseous metabolism of the heart, oxygen and CO_2 determinations were carried out in a manner which will be briefly described.

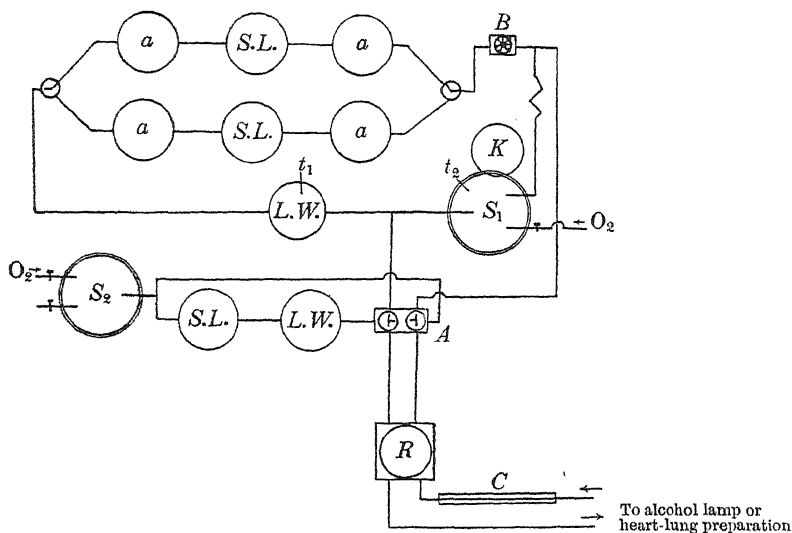


Fig. 1. Diagram of apparatus. *A*, stopcocks for connecting or disconnecting alcohol lamp or isolated heart-lung preparation from the recording system; *B*, rotary blower; *S*₁, spirometer with *K*, kymograph for record of oxygen consumption; *S*₂, reserve spirometer; *R*, respiration pump; *SL*, soda lime bottle; *a*, acid bottle; *LW*, lime water bottle; *C*, condenser; *t*₁, *t*₂, thermometers reading to 0.1° C.

Description of apparatus. An examination of Fig. 1 and its legend will show the general arrangement of apparatus. It will be seen that there are two spirometers each with a CO_2 absorbing system, with either of which the lungs of the animal may be connected by stopcocks *A*. In the case of a mammalian experiment this permits of efficient oxygenation of the blood with a removal of CO_2 , thus allowing the blood CO_2 content to fall to a steady low level before commencing the experiment. It will be noted that when the animal is turned into the system associated with

spirometer S_1 , no expired air can enter the spirometer. In order to ensure this, the tube marked D , leading from the spirometer to the rotary blower, is of 300 c.c. capacity. A large portion of the expired air passes into this tube before being passed on by the rotary blower B , simply because the velocity of the expiration due to the elasticity of the lungs with a respiration rate of eighteen per minute and a tidal air volume of 150 c.c. may be as high as 200 c.c. per sec. It is unnecessary to circulate the air at a rate of 28 to 30 litres per minute; 7 to 12 litres per minute is ample and allows of a complete absorption of CO_2 .

The temperature is noted by thermometers reading to 0.1°C . in Williams's bottles, t_1 , t_2 , and in the cannula which passes the blood to the heart. Soda-lime is kept in Williams's bottles, not in the usual soda-lime bottles, which have been found unsatisfactory. When the Williams's bottles with soda lime and acid have been weighed and placed in position the rotary blower is started, and the air in the system with spirometer S_1 circulated for 15 minutes to bring it to a constant temperature. Meanwhile the lungs are being artificially inflated by a Starling Ideal pump placed in oil, the air being circulated through a CO_2 absorbing system and spirometer S_2 which has previously been filled with oxygen. When the temperature at t_1 is steady the lungs are switched into connection with S_1 by means of the stopcocks A , the kymograph and stop-watch are started and the temperatures noted. At any time the circulating air can be turned into the parallel absorbing system and a new period started.

Alcohol lamp check experiments on the apparatus. Absolute ethyl alcohol of 99.95 p.c. purity and having a sp.gr. of 0.794 was used. The oxygen equivalent of the alcohol was, 1 c.c. = 1159.60 c.c. of O_2 . The factor for the large spirometer bell is calculated from the steel mould from which it is made. This mould had a diameter of 16.25 cm., which gives a factor of 20.73 c.c. per mm. excursion of the bell. For the estimation of small amounts of oxygen consumed by the heart we had made for us a very finely balanced spirometer, the bell of which is 7.55 cm. diameter and 34 cm. in height, having a factor of 4.461 c.c. per mm.

The system as described, and as shown in Fig. 1, was tested for leaks by recording movements of the spirometer bell for 15 minutes, in which time the recording kymograph drum made one complete revolution.

Respiratory quotients with the alcohol lamp. Reference to Table I will show the details of ten check experiments. The experimental error for the estimation of oxygen consumed averages 0.667 p.c., that for R.Q.'s averages 0.225 p.c. In the case of organs where the amounts of oxygen utilized per minute is very small it is possible to secure, with the smaller

spirometer, a continuous and very accurate record of oxygen utilization over periods extending from 1, 2 or more hours, depending upon the gradient of the curve of oxygen consumption.

TABLE I. R.Q. check experiments with alcohol lamp.

No.	Alcohol c.c.	Spiro- meter mm.	t_2 change ° C.	t_1 change ° C.	Theore- tical O ₂ c.c.	Exp. O ₂ c.c.	Per- centage O ₂ rec.	CO ₂ c.c.	R.Q. exp.
1	1.42	85	0.20	0.20	205.90	205.53	99.71	136.82	0.665
2	1.76	107	0.20	0.00	132.10	131.65	99.65	87.94	0.668
3	2.45	154	0.04	0.10	189.46	193.01	101.87	130.85	0.677
4	2.78	172	0.00	0.12	214.98	214.65	99.89	145.88	0.678
5	1.75	111	0.10	0.05	203.00	205.48	101.02	136.38	0.664
6	1.42	90	0.05	0.10	205.90	210.60	102.27	143.10	0.675
7	2.20	157	0.00	0.02	216.92	217.15	100.10	144.08	0.664
8	1.90	118	0.20	0.03	220.40	220.89	100.22	146.05	0.661
9	2.03	129	0.03	0.00	235.48	239.35	101.64	158.77	0.663
10	1.85	115	0.00	0.00	214.61	215.28	100.31	143.50	0.666

Time of experiments 10 min. by stop watch, except Nos. 1 and 6 = 8 min.
Nos. 2 and 3 = 15 "

Average R.Q. = 0.6681

Theoretical = 0.6666

Average p.c. O₂ recovered = 100.66

The isolated heart-lung preparation needs no description, suffice it to add that the reservoir, in which the blood is usually exposed to the external air, was effectively closed by being connected to a small spirometer filled with nitrogen. The fall in the bell of this spirometer indicated with extreme accuracy any alteration in the blood volume of the preparation.

Sugar estimations were made by the Shaffer and Hartmann [1920] method, lactic acid by West's modification of the method described by Friedemann, Cotonio and Shaffer [1927]. Numerous experiments have been carried out and, as these entail the presentation of many figures, only two or three typical examples of each will be given. For convenience in discussing the subject-matter the work will be presented under the following divisions.

Part I. *Hyperglycemia with and without insulin.*

- (1) The utilization of sugar.
- (2) The respiratory quotient and oxygen consumption.
- (3) The synthesis of glycogen.

Part II. *Hypoglycemia with and without insulin.*

- (1) The utilization of sugar.
- (2) The respiratory quotient and oxygen consumption.
- (3) The utilization or synthesis of glycogen.

PART I. HYPERGLYCÆMIA.

(1) *The utilization of sugar.*

In discussing the question of sugar utilization it is essential not to confuse sugar utilization by the heart with the amount of sugar disappearing from the circulating blood. The early experiments of Locke and Rosenheim [1907], Knowlton and Starling [1912*a*], Mansfield [1914], Patterson and Starling [1913], and later experiments of Hepburn and Latchford [1922], Burn and Dale [1924], of Cruickshank and Shrivastava [1930] and of many others have shown that the mammalian heart removes sugar from the blood, the figures, under varied experimental conditions, ranging from 2 to 6 mg. per g. of heart muscle per hour. In using the isolated heart-lung preparation to determine sugar utilization by the heart, Starling and Evans [1914] allowed 1.3 mg. per g. of heart per hour for the sugar consumption of the lungs and the blood. Cruickshank and Startup [1930] place the sugar consumption of the dog's lungs at 0.7 to 0.8 mg. per g. of heart muscle per hour. The rate of disappearance of sugar from circulating well-oxygenated blood has been investigated by Cruickshank and Startup [1932] and they have shown that a progressive oxidation of sugar takes place amounting to approximately 10 p.c. of the total blood sugar in the first hour and about 14 p.c. in the second hour. No glycolysis takes place in this time, provided oxygenation is good. It would seem, therefore, from these facts that a correction of 1 mg. per g. of heart muscle per hour should be made for utilization of the lungs in determining absolute figures for the sugar usage of the heart. This correction has not been made in the tables presented in this paper.

In hyperglycæmia without insulin the utilization of sugar is increased from an average normal of 5.28 to 5.84 mg. per g. of heart muscle per hour. The addition of insulin increases the sugar consumption to the average figure of 6.06 mg. per g. per hour. In these experiments the blood-sugar level was never in excess of 0.218 g. p.c. Where the sugar content is increased to 0.40 g. p.c. or more there is not a proportional increase in the utilization under insulin. The addition of sugar to the blood whereby a moderate condition of hyperglycæmia is maintained raises sugar oxidation 10.5 p.c.

The effect of insulin. The addition of ten units of insulin per hour has a much less effect than excess sugar in stimulating sugar oxidation (Table II); the average percentage increase being 3.62. In any discussion of sugar utilization, when the disappearance of blood sugar is in question,

TABLE II. Hyperglycæmia.

The effect of hyperglycæmia and of added insulin upon the
R.Q. oxygen consumption and sugar oxidation.

Hour of exp.	Blood sugar g. p.c.		CO ₂ c.c.	O ₂ c.c.	R.Q.	Oxygen c.c. per g. per hr.	Sugar oxi- dized mg. per g. per hr.	Lactic acid mg. p.c.		
	Begin	End						Begin	End	
1	0.1128	0.0859	362.29	357.71	1.012	4.47	5.97	31.5	28.8	Normal
2	0.1701	0.1419	381.68	375.67	1.016	4.70	6.25	31.5	27.0	Insulin
1	0.1432	0.1008	302.51	273.40	1.106	4.43	5.78	54.9	55.8	Normal
2	0.1863	0.1244	279.89	283.50	0.980	4.50	6.00	55.8	58.5	Insulin
1	0.1708	0.1206	361.31	363.45	0.999	4.33	5.78	27.2	28.8	Normal
2	0.2218	0.1244	369.99	375.40	1.018	4.46	5.93	31.5	27.9	Insulin
Average										
	—	—	—	336.60	—	3.96	5.28	Normal, 6 exps.		
	0.1422	0.1039	342.04	331.55	1.039	4.376	5.84	With sugar		
	0.1927	0.1319	343.85	344.85	1.004	4.553	6.06	With insulin		
	Increase due to sugar					10.50	10.50			
	Increase due to insulin					3.62	3.62			

one must clearly discern between oxidation and synthesis. As this involves a discussion of both sugar and oxygen consumption in the presence of insulin and also of the direct action of insulin on the heart muscle, the matter will be fully referred to under the section dealing with the effect of insulin upon the oxygen consumption of the heart in hypoglycæmia.

(2) *The respiratory quotient and oxygen consumption.*

The normal isolated heart in the presence of an adequate supply of blood sugar has always given a R.Q. which is unity. Bayliss, Müller and Starling [1928] find a R.Q. for the isolated heart of 0.90, a figure determined after 3 hours have been spent upon the operation and on the setting up of the apparatus. In our experiments the preparation is completed in 15 to 20 minutes and runs for periods of 1, 2 or 3 hours. The average figures for the utilization of oxygen (Table II) show how small is the effect, first of added sugar and second of sugar plus insulin. With a maintained blood sugar the heart consumes approximately 4 c.c. O₂ per g. of heart muscle per hour. Upon the addition of sugar, raising the percentage to an approximate level of 0.15 g., the oxygen consumption per g. per hour rises to an average of 4.376 c.c. while the subsequent addition of ten units of insulin raises the figure to 4.553 c.c. per g. per hour, an increase of 3.62 p.c. As with added sugar so with insulin; the increase in oxygen consumed is determined by the amount

of sugar utilized. Figures for the lactic-acid content of the blood show that changes in the production of lactic acid are of such a small order in the first experimental hour that they cannot materially alter the results obtained on the oxygen consumption and sugar utilization by the isolated heart in hyperglycæmia.

(3) *Glycogen synthesis.*

This was determined by correlating the blood sugar lost with the sugar equivalent of the oxygen consumed.

With the maintenance of the blood-sugar level above the normal there is a slight increase in the deposition of glycogen in the heart. Upon the addition of insulin there is a marked increase in the amount of sugar deposited as glycogen in the heart. Table III shows that of the

TABLE III. Hyperglycæmia.

Effect of insulin on the absolute and percentage amounts of blood sugar oxidized and stored by the mammalian heart.

(Blood-sugar percentages and E.Q.'s are shown in Table II.)

Exp.	Total sugar lost g.	Sugar oxidized g.	Sugar stored g.	Per- centage blood sugar oxi- dized g.	Per- centage blood sugar stored g.	Wt. of heart g.	Possible glycogen in heart g.	Percentage increased in heart glycogen	
I	0.4907	0.4769	0.0138	97.2	2.8	80	0.400	3.5	Normal
	0.6405	0.5008	0.1397	78.2	21.8		0.414 0.553	35.0	Insulin
II	0.3752	0.3644	0.0108	97.2	2.8	63	0.315	3.1	Normal
	0.5054	0.3780	0.1274	74.8	25.2		0.325 0.452	39.1	Insulin
III	0.5004	0.4858	0.0146	97.1	2.9	84	0.420	3.3	Normal
	0.6564	0.5005	0.1560	76.4	23.6		0.434 0.590	36.2	Insulin

The percentage increase in the oxidation of sugar and the deposition of glycogen in the heart in hyperglycæmia with and without insulin (Tables II and III).

Hyperglycæmia alone		Hyperglycæmia with insulin	
Oxidation	Glycogen	Oxidation	Glycogen
10.5	3.30	3.62	36.70

The effect of hyperglycæmia with and without insulin upon the balance between the oxidation and synthesis of 100 parts of blood sugar.

Hyperglycæmia alone		Hyperglycæmia with insulin	
Oxidation	Synthesis	Oxidation	Synthesis
97.2	2.8	76.1	23.5

total amount of sugar which has disappeared from the blood, the amount deposited has increased from 2.8 to 23.5 p.c. These results are even more striking when we consider the relation between the total amount of blood sugar used for oxidation and that used for the synthesis of glycogen. Taking average figures; the amount of sugar oxidized increases from 0.4433 to 0.4736 g., an increase of 0.0313 g., that used for the synthesis of glycogen increases from 0.0131 to 0.1442 g., an increase of 0.1311 g. The former figure shows a negligible increase, the latter a tenfold increase.

If we assume that the heart muscle has an initial glycogen content of 0.500 g. p.c., estimated as sugar, we see from Table III that there has been an average increase from 3.3 to 36.7 p.c. These figures for glycogen deposition, which are in terms of the original amount of glycogen in the heart, are in remarkable agreement with the findings of Cruickshank and Shrivastava [1930] who found, by numerous direct estimations of heart glycogen in dogs, that at the end of 2 hours' insulin administration with a blood sugar not greater than 0.222 g. p.c., there was an average change from 0.535 to 0.719 g., a 34.3 p.c. increase.

From a consideration of such results it is fair to conclude that insulin has, as far as cardiac muscle is concerned in the presence of hyperglycæmia, a very marked stimulating effect upon glycogen synthesis, and plays but a small part in increasing sugar oxidation. It is also of interest under these conditions to note the effect of insulin on the percentage distribution of blood sugar between oxidation and synthesis. Normally the ratio is 97.2 to 2.8; with insulin it becomes 76.1 to 23.5, a ratio which is noteworthy, as will appear later.

PART II. HYPOGLYCÆMIA.

(1) *Utilization of sugar.*

It may be inferred that the heart is faced with a condition of hypoglycæmia when the blood sugar is not sufficient to supply fully the energy requirements of the organ, and as a result thereof a certain amount of glycogen has been utilized. This condition of affairs invariably happens when the blood sugar has been reduced to 0.05 g. p.c. In an isolated heart-lung preparation with a blood sugar of 0.0935 g. p.c. at the beginning and 0.0546 g. p.c. at the end of 1 hour's experiment, the amount of sugar utilized averages 4.55 mg. per g. of heart muscle per hour (Table IV). But the amount of sugar disappearing from the blood is not sufficient for the energy needs of the heart, with the result that there is a call upon the glycogen content of the heart. It can be seen from Table V that the

Table IV. Hypoglycæmia.

Effect of hypoglycæmia upon the R.Q., the oxygen consumption and sugar oxidation in the mammalian heart. Time = 1 hour.

Blood sugar g. p.c.		CO ₂ c.c.	O ₂ c.c.	R.Q.	Oxygen c.c. per g. per hr.	Sugar oxidized mg. per g. per hr. g.	Lactic acid mg. p.c.	
Begin	End						Begin	End
0.0979	0.0572	278.89	280.35	0.992	3.50	4.67	40.5	43.6
0.0928	0.0599	336.07	339.12	0.991	3.02	4.04	45.0	49.5
0.1121	0.0620	473.14	468.00	1.011	3.60	4.80	36.0	41.4
0.1009	0.0712	549.60	554.41	0.996	3.46	4.62	37.8	42.3
0.1019	0.0488	509.79	524.37	0.972	3.49	4.66	57.6	59.4
Average								
0.0935	0.0546	434.10	425.61	0.992	3.42	4.55		
Normal of 6 expts.		—	336.60	—	3.96	5.28		
Percentage fall below normal			—	—	13.90	13.90		

TABLE V. Hypoglycæmia.

Effect of hypoglycæmia on the glycogen content of the heart.
(Blood-sugar percentages and R.Q.'s are shown in Table IV.)

Exp.	Blood sugar lost g.	Total sugar oxidized g.	Percentage		Glycogen lost g.	Weight of heart g.	Possible heart glycogen g.	Per- centage glycogen lost
			sugar added to blood sugar for oxidation					
I	0.2584	0.3738	44.6		0.1154	80	0.400	28.8
II	0.3342	0.4521	30.9		0.1184	112	0.560	21.1
III	0.5608	0.6240	11.3		0.0636	130	0.650	9.7
IV	0.5961	0.7392	23.9		0.1431	160	0.800	17.8
V	0.4828	0.6858	42.0		0.2030	150	0.750	27.0

Sugar added to blood sugar from heart reserves = 30.54 p.c.

Average loss of glycogen in first hour = 20.88 p.c.

blood sugar used has been augmented 11.3 to 44.6 p.c. by a call upon the carbohydrate reserve of the heart muscle. It is, of course, impossible to determine the glycogen content of the heart at the commencement of the experiment, but it is of interest to note the loss or gain of glycogen assessing the original heart glycogen from the assumption that the normal heart contains 0.500 g. p.c. of its weight as glycogen. It is an assumption based on the average results of numerous experiments dating from the year 1912; reference has already been made to the close parallel between these figures and those obtained from direct estimation of heart glycogen. On this assumption it is seen that there is a glycogen loss varying in five experiments from 9.7 to 28.8 p.c., with an average loss of 20.88 p.c. From such results one is led to the conclusion that the heart, when faced with a slight hypoglycæmia, draws upon its carbohydrate reserves

and, as will be seen later when the R.Q.'s are discussed, upon nothing else, at least not as far as can be judged from an experiment lasting only 1 hour. This is not in accord with what obtains in the eviscerated spinal cat, where a slight degree of hypoglycæmia is not accompanied by any marked loss in the glycogen of the skeletal muscle [Best, Hoet and Marks, 1926]. In the eviscerated preparation, however, liver glycogen may have been available, the blood-sugar level was never lower than 0.068 g. p.c., and no estimation of heart glycogen was made.

The effect of insulin. It has been seen that, when no sugar is added to maintain a constant blood-sugar level, the sugar utilization falls from the

TABLE VI. Hypoglycæmia.

The effect of insulin on the R.Q., oxygen consumption, and sugar utilization of the heart.

Hour of exp.	Blood sugar g. p.c.		CO ₂ c.c.	O ₂ c.c.	R.Q.	Oxygen c.c. per g. per hr.	Sugar oxidized mg. per g. per hr.		In-sulin units	Lactic acid mg. p.c.	
	Begin	End								Begin	End
1	0.1024	0.0592	356.23	353.63	1.007	4.21	5.61	10		34.2	36.9
2	0.0592	0.0246	346.05	360.50	0.960	4.29	4.74	10		36.9	43.2
3	0.0246	0.0246	277.08	357.48	0.775	4.25	1.42	10		43.2	40.5
1	0.1091	0.0605	381.57	352.30	1.082	4.14	5.52	10		45.0	40.5
2	0.0605	0.0447	305.34	356.40	0.860	4.20	2.85	10		40.5	36.9
3	0.0447	0.0313	264.44	356.11	0.744	4.20	0.72	10		36.9	22.5
1	0.0977	0.0413	356.23	364.50	0.988	4.18	5.58	10		27.0	27.0
2	0.0413	0.0261	305.34	380.75	0.802	4.38	1.83	10		27.0	24.0
1	0.1009	0.0712	549.60	554.41	0.996	3.46	4.62	0		37.8	42.3
2	0.0712	0.0506	503.80	574.12	0.877	3.58	2.77	10		42.3	25.2
1	0.1019	0.0488	509.79	524.37	0.972	3.49	4.66	0		57.6	59.4
2	0.0673	0.0315	492.13	550.88	0.910	3.66	3.47	10		54.9	41.4

Note. (1) In the two last experiments insulin was given at the commencement of the second hour.

(2) The amount of sugar oxidized for R.Q. given has been determined from the tables of Zuntz and Schumberg.

Average figures.

Normal of 6 expts. 336.60 — 3.96 5.28

Average weight of heart = 85.4 g.

Hypoglycæmia with insulin from the beginning of the experiment.

First hour:

Commencing blood sugar normal 1.028 4.17 5.56

Second hour:

With very low blood sugar 0.881 4.30 3.09

Third hour:

0.760 4.24 1.07

Percentage changes in the amount of oxygen used and sugar oxidized in hypoglycæmia with insulin.

	Oxygen used	Sugar oxidized
First hour	+5.30	+ 5.30
Second hour	+3.12	-44.42

normal of 5.28 to 4.55 mg. per g. per hour, a depression of 13.90 p.c. The addition of insulin at the beginning of the experiment when the blood sugar is normal results in a slight increase of sugar utilization to 5.56 mg. per g. per hour, an increase of 5.30 p.c. (Table VI). If, however, the insulin be added at the end of the first hour of the experiment, when a definite degree of hypoglycæmia has become established, the effect is a decrease in sugar consumption as shown in the last two experiments in Table VI. This is corroborated by what obtains when insulin is added hour by hour; the amount of sugar used becomes less and less, until finally little sugar if any may be utilized. Table VII shows this result; after a slight initial rise in the first hour, sugar utilization falls 44.42 p.c.

TABLE VII. Hypoglycæmia.

The effect of insulin on the oxidation and storage of blood sugar by the mammalian heart. (Blood-sugar percentages and R.Q.'s as in Table VI).

Hour of exp.	Total sugar lost g.	Blood sugar oxidized g.	Blood sugar stored g.	Per-centage of sugar oxidized	Per-centage of sugar deposited g.	Weight of heart g.	Possible heart glycogen g.	Per-centage gain heart glycogen
1	0.5332	0.4712	0.0620	88.38	11.62	84	0.420	14.80
2	0.4482	0.3985	0.0497	88.91	11.09	—	0.482 0.532	10.31
1	0.5263	0.4696	0.0567	89.23	10.77	85	0.425	13.34
2	0.2475	0.2204	0.0271	81.52	19.48	—	0.481 0.508	5.58
1	0.5367	0.4666	0.0701	86.94	13.06	87	0.435	16.10
2	0.1788	0.1589	0.0199	88.78	11.22	—	0.506 0.524	3.54
2	0.5246	0.4436	0.0810	84.56	15.44	160	0.656 0.737	12.34
2	0.5877	0.5210	0.0667	88.65	11.35	150	0.547 0.614	12.19

The percentage changes due to hypoglycæmia with and without insulin in the oxidation processes and the deposition of glycogen in the heart (Tables VI, VII).

	Hypoglycæmia alone		Hypoglycæmia with insulin	
	Oxidation	Glycogen lost	Oxidation	Glycogen gained
First hour	-13.90	-20.88	5.30	13.75
Second hour	—	—	3.12	6.48

The effect of hypoglycæmia with and without insulin upon the ratio between the oxidation and synthesis of 100 parts of blood sugar.

	Hypoglycæmia alone		Hypoglycæmia with insulin	
	Oxidation	Synthesis	Oxidation	Synthesis
First hour	100.00	0.00	87.55	12.45
Second hour	—	—	86.17	13.93

in the second hour and 66.11 p.c. in the third hour. Third-hour results have not been included in the tables because the onset of lung oedema makes them unsatisfactory. When the blood sugar is reduced to about 0.025 g. p.c. the heart has apparently ceased to make use of sugar as its chief source of energy. That there is a condition of glucatonia is clear from the very small amount of sugar in the serum at the end of the third experimental hour. Such a diminution of sugar in the serum points to an extremely small amount of free muscle sugar, proof of a thorough removal of sugar by the heart. The question arises now as to whether the sugar so removed has been oxidized or synthesized.

(2) *The respiratory quotient and oxygen consumption.*

The respiratory metabolism of the isolated mammalian heart can best be investigated by the isolated heart-lung preparation of Starling [Knowlton and Starling, 1912*b*], for here the heart can be kept beating powerfully and efficiently for several hours with both the circulating and respiratory systems closed from the external air.

"If the heart muscle, like skeletal muscle, performs its work by oxidising preformed carbohydrate exclusively then the respiratory quotient should be unity" [MacLeod, 1928]. Lovatt Evans [1912] found great variations in the R.Q. of mammalian hearts, the average for dogs being 0.901. Starling and Evans [1914] obtained quotients as low as 0.70 and as high as 1.09 with an average of 0.85. It has been assumed, because of such results, that important metabolic differences exist between skeletal and cardiac muscle.

A high R.Q. may be due to the washing out of CO_2 in such experiments [Kilborn, 1928], but this factor has been controlled in the experiments discussed here by adjusting the extent of ventilation to the size of the preparation [Corkhill, Dale and Marks, 1930; Eggleton and Evans, 1930], maintaining a much more moderate degree of ventilation than is usual in such experiments and by allowing at least half an hour for complete gaseous equilibrium to be obtained before commencing the actual experiment. With a ventilation rate which was generally constant at twelve strokes per minute, the stroke volume, which varied between 50 and 100 c.c., was changed in accordance with the size of the heart and lungs. Over-ventilation was thus guarded against, and the small amount of tissue used permitted of a fairly quick arrival at gaseous equilibrium. No anoxæmia was ever present, a perfect oxygenation of the blood was always in evidence to the end. That there was no failure in oxidative processes in the preparation is also seen from the fact that there was little

increase of lactic acid in the blood, in fact, in the insulin experiments there was usually a marked diminution in the lactic acid content of the blood.

The effect of hypoglycæmia per se on the respiratory metabolism of the heart. Table IV shows that the R.Q. for the first experimental hour is essentially unity, the highest figure being 1.011, the lowest 0.991, with an average of 0.992. A moderate degree of hypoglycæmia has therefore had no effect upon the type of metabolic activity. The oxygen consumption is also without marked variations when reduced to c.c. per g. of heart muscle per hour. The lactic acid of the blood varied in these experiments from 36 to 59 mg. p.c. The variation in each experiment is so small as to be regarded as negligible as far as any change in oxidative processes may be concerned. Hypoglycæmia produced a diminution in the utilization of oxygen from 3.96 to 3.42 mg. per g. of muscle per hour, an average fall of 13.90 p.c.; sugar utilization has also been reduced to a similar extent from 5.28 to 4.55 mg. per g. of heart muscle per hour. The normal figures are from the records of six experiments in which the sugar level was maintained by the use of a Master's constant injection pump.

The effect of insulin. It has been shown that, with an increase in the sugar content of the blood above the normal level for the animal, there is a slight increase in oxygen consumption. The addition of insulin in hyperglycæmia effects a further slight increase in the oxidation of sugar by the heart, but the oxygen absorbed does not account for all the sugar which has disappeared; much of it has been retained as glycogen. Experiments by Burn and Dale [1924] upon eviscerated decapitated cats led them to the conclusion that the earlier stages of insulin action are accompanied by increased consumption of oxygen in a preparation, the R.Q. of which was always about unity.

In a later paper Best, Dale, Hoet and Marks [1926*a*] state that there is always a slight depression of respiratory metabolism following insulin administration which, calculated from only one experiment, amounts to 5.4 p.c. of the oxygen figure for the normal period, and they say that "the excess oxygen consumption observed by Burn and Dale [1924] can be entirely accounted for by the contribution to the blood from the carbohydrates of the liver." But a recalculation of Burn and Dale's results will show that, after allowing for the leakage of sugar, the increased amount oxidized in the presence of insulin varies from 9.1 to 43.8 p.c. with an average figure of 23.4 p.c. or, leaving out the extreme figure of 43.8 p.c., with an average figure of 16.3 p.c. Experiments of Chaikoff and MacLeod [1927] have shown that insulin causes a relatively slight increase in the oxidation of carbohydrates in the

rabbit but a definite increase in the isolated perfused muscles of the cat. They also point out that in dogs there is also a very slight increase in oxygen consumption when insulin is administered with glucose. A marked increase in oxygen consumption by animals to which insulin has been given has been thought to be due to a condition of hyper-excitability of muscle tissue which preceded the onset of convulsions [Dickson, Eadie, MacLeod and Pember, 1924]. On the other hand, Visscher and Müller [1927] have demonstrated that non-pressor samples of insulin produce no direct stimulation of oxidative metabolism in the heart.

It is clear from what has been said that one should determine whether or not insulin has any direct effect upon the preparation used, be it a whole animal or an isolated tissue. Since the discussion here is one of synthesis versus oxidation as characteristic of insulin action, pressor effects must either be eliminated or accounted for. In the isolated heart-lung preparation the heart suffers no change in rate when twenty units of insulin are added to the blood; there is, however, a slight rise in blood-pressure which never exceeds 10 mm. Hg, and which passes off within 10 to 15 minutes. Further it is seen that there is, during this time, a slight increase of 20 to 30 c.c. in the volume of blood in the reservoir, which is due to a reduction in the blood volume of the preparation, the volume returns to its pre-insulin level within 15 minutes. Here then is evidence of a slight and transient pressor effect upon isolated heart muscle. To make a correct allowance in the figure for oxygen consumption for this pressure change one must take into consideration its duration. A 10 p.c. rise in pressure disappearing in 10 minutes would necessitate a correction for the figures given of approximately 1.7 p.c. This correction has not been made in the tables.

Experiments were carried out on the whole animal (two dogs and three cats) to see to what extent oxygen consumption would be increased in the presence of forty units of insulin. In the cats the oxygen consumption increase averaged 7.69 p.c. in 1 hour, there was a very slight increase of 5 mm. Hg in blood-pressure, the increase in heart rate averaged 1.8 p.c. In the dogs there was no change in blood-pressure, an average increase in heart rate of 1.7 p.c. and an average increase in oxygen consumption of 2.7 p.c. A repetition, within half an hour of the first dose, of forty units of insulin caused a slight fall in blood-pressure. Pressor effects due to the insulin can therefore be regarded as negligible. Dr K. K. Chen of Eli Lilly and Co. very kindly tested all the samples of insulin which he sent to us and found that they caused no rise of blood pressure in the pithed cat.

Insulin given in ten unit doses hourly in hypoglycæmia raises oxygen consumption 5·30 p.c. in the first hour (Table VII). In the second hour there is a continued slight rise in oxygen consumption, and in the third hour there is no appreciable change. Further, insulin addition generally produces a definite fall in the lactic-acid content of the blood which, with a slight increase in oxygen used, a decrease in CO_2 produced, and a fall in the R.Q. would indicate that the lactic acid is not oxidized but is reconverted to glycogen. The slight increased oxidation must therefore be attributed to a consumption by the heart of non-carbohydrate substances. From such results one must conclude that the stimulation of oxidative processes is not essentially a characteristic of insulin action.

The most noteworthy change, however, is that seen in the R.Q. A fall in the R.Q. from 1·00 to 0·744 (Table VI) indicates a definite change in metabolism. As no measure of the extent of protein metabolism has been obtained and as a correction at a R.Q. of 0·82 would entail approximately a 10 to 15 p.c. change which would not materially affect the results as set down, the R.Q.'s have been taken as representing the balance between carbohydrate and fat in so far as figures for the oxidation of sugar have been given. The relative proportion of the oxygen consumed by sugar has been obtained from the well-known tables of Zuntz and Schumburg. It may be taken, from a consideration of the fall in the R.Q.'s, that the energy cost of the work that is being done by the heart is greater since the calorific output as estimated by oxygen consumption is slightly raised and maintained over a period of 3 hours, and the metabolic activity of the heart muscle has changed in type, namely from a purely carbohydrate source of energy to one consisting to a large extent of non-carbohydrate material.

(3) *The utilization or synthesis of glycogen.*

It has been clearly shown by Best, *et al.* [1926*a*] and Burn and Dale, [1924], Cori, Cori and Pucher [1923], Bissinger, Lesser and Zipf [1923], Cruickshank and Shrivastava [1930] that the chief action of insulin is to cause increased glycogen formation in the presence of an adequate supply of blood sugar. The observations of Bissinger, *et al.* and of McCormick and MacLeod [1923] showed that, while glycogen is deposited as an early action of insulin, little if any excess remains after a certain period. Dudley and Marrian [1923] concluded as a result of their experiments that hypoglycæmia due to insulin produced a definite glycogenolysis in liver and muscle but an increase in glycogen within the heart muscle. It has, however, been clearly shown [Best, Hoet and

Marks, 1926*b*] that hypoglycæmia with or without insulin does not, unless convulsions supervene, produce any breakdown in the glycogen of skeletal muscles. In the eviscerated spinal preparation the blood sugar is evidently sufficient for the energy needs of the preparation, with the result that a certain small amount of glycogen is stored. Upon the addition of insulin with a blood-sugar level at the end of the experiment of 0.06 g. p.c. there is an increase in glycogen to the extent of 28 p.c. [Best, *et al.* 1926*a*].

Reference has already been made to the loss of heart glycogen occasioned by a marked hypoglycæmia, namely an augmentation of blood sugar from heart glycogen averaging 30.54 p.c. or an average of 20.88 p.c. of the calculated glycogen content of the heart being utilized. This loss is progressive and may with a continued hypoglycæmia be as much as 30 p.c. of available glycogen at the end of 2 hours. It would be of interest to determine the ultimate reaction of the isolated mammalian heart when faced with practically no blood sugar, and a 50 or 60 p.c. depletion of its glycogen.

The effect of insulin. The results shown in Table VII indicate that in insulin hypoglycæmia lasting 2 to 3 hours there is such a depletion of blood sugar that further sources of energy are necessary. It should also be remembered that the depletion of sugar may be greater than the figures would indicate, because the method of estimation of blood sugar has not excluded non-carbohydrate reducing substances [Somogyi, 1930]. In the first hour, with a R.Q. of unity, of the total amount of blood sugar which has disappeared, an average of 12.5 p.c. has been deposited in the heart muscle, in the second hour 13.93 p.c. In terms of the assumed original amount of glycogen in the heart the increase in glycogen is, in the first and second hours, 13.75 and 6.48 g. p.c. This shows that in a progressive insulin hypoglycæmia while the blood is rendered almost sugar free, and the amount of glycogen deposited in the heart has accordingly fallen, yet the ratio distribution between sugar oxidized and sugar synthesized is remarkably well maintained. It is apparent that insulin effects a balance between these two activities and that in favour of synthesis, and further, that it continues to do so when the heart exposed to an extreme degree of hypoglycæmia is forced to utilize other substances as sources of energy.

It is thus demonstrated that, what can be easily obtained by skeletal muscle under much more favourable conditions than those afforded the isolated heart, has been attempted, and that with considerable success, by cardiac muscle.

DISCUSSION.

It would appear from such results that, while carbohydrate is available to meet the increased energy needs of the heart muscle, carbohydrate only is used, but, that with the maintained energy requirements and with a rapid depletion of sugar supplies the heart muscle is forced to fall back on other sources of energy. That in its early stages exercise may be accomplished by the metabolism of carbohydrate has been shown by Hill [1925] and his co-workers, and that strenuous and continued exercise demands that the musculature of the body fall back on fat has been shown by Furusawa [1926].

It has been suggested by Greene [1926], from observations on the glycogen content of the muscle of salmon, that carbohydrate is not the immediate fuel of muscular activity; but that carbohydrate is the chief source of the energy of muscular contraction has been demonstrated by the work of Meyerhof [1924] and Hill [1922, 1932]. Burn and Dale [1924] found that the skeletal muscle of the eviscerated preparation before and after insulin had a R.Q. close to unity, and suggested therefrom that carbohydrate is the chief source of energy of isolated muscle.

On the other hand Kilborn [1928] offers no support to the theory that carbohydrate is the only source of energy for muscle contraction, and he attributed the high R.Q. of Burn and Dale to a washing out of CO_2 . Bornstein [1929] showed that, in the eviscerated dog, a R.Q. of unity was maintained provided the blood-sugar level was kept up, but that it fell with a diminution in the carbohydrate supply. Corkhill, Dale and Marks [1930] have more critically investigated the question of over-ventilation as affecting the R.Q. in the eviscerated cat, and they state that a R.Q. of unity should be regarded as correct for that preparation provided the blood sugar is maintained at the normal value, and further they affirm that such a quotient cannot be regarded as artificially produced by excessive ventilation.

Meyerhof and Boyland [1931] have shown that skeletal muscle poisoned with iodoacetic acid and having a R.Q. of 0.7 to 0.8 recovers in oxygen with no lactic acid and with but little carbohydrate oxidation. The observations of Meyerhof [1931] and those of Witting, Markowitz and Mann [1930], on the isolated rabbit's heart perfused with glucose-free Ringer-Locke's solution, which show that an almost glycogen-free heart will beat as long as the normal perfused heart, would tend to strengthen the view that the work of muscular contraction may be performed by the oxidation of material other than carbohydrate. More

recent work by Clark, Gaddie and Stewart [1932] shows that frog's heart perfused for 6 hours under aerobic conditions gives a R.Q. of 0.87, from which they conclude that carbohydrate and protein would form 40 and 60 p.c. respectively of the total metabolism.

The results of our experiments on cardiac metabolism under hyperglycæmia and hypoglycæmia lead us to the conclusion that as with skeletal muscle so is it with mammalian heart muscle; given an adequate supply of carbohydrate the R.Q. will be in the region of unity, with a diminution of carbohydrate supplies the R.Q. will accordingly fall, indicating a definite change in the type of metabolism. While the question of fat and protein utilization is beyond the scope of this paper, still it would appear that when carbohydrate is not readily available as in hypoglycæmia then the heart must needs fall back on protein and fat, a view in keeping with the newer ideas as put forward by Embden and Meyerhof on the chemistry of skeletal muscle contraction.

It is further evident that, for the increase of glycogen in the heart in hyperglycæmia as for its restoration in hypoglycæmia, insulin is essential. This conclusion is in keeping with results obtained on skeletal muscle [Dale and Burn, 1924; Debois, 1930, 1931] and on dehepatized animals [Markowitz, Mann and Bollman, 1929]. It makes more difficult of explanation the fact that the diabetic heart contains more glycogen than the normal [Cruickshank, 1913] and that insulin does not lead to any increase in glycogen in the diabetic heart [Cruickshank and Shrivastava, 1930]. It is probable that glycogen synthesis is dependent upon a balance between sugar concentration and insulin activity.

The addition of insulin increases the call upon the blood sugar and, were it not for the fact that the function of synthesis is intensified by insulin, it is possible that the carbohydrate reserve of the heart would be the more readily available. While there is here no evidence for the assumption it may also be that insulin has stimulated gluconeogenesis from protein and fat within the heart muscle.

It would appear therefore from these experimental results that the immediate effect of insulin is to raise, very slightly, oxygen consumption and to increase markedly glycogen synthesis and to do so at the expense of blood sugar. This conclusion is inevitable in view of a R.Q. of unity and a definite degree of glycogen synthesis as shown in Tables VI and VII.

The continuance of insulin with the production of a definite hypoglycæmia, while it maintains oxygen consumption and glycogen synthesis, is associated with a marked fall in the amount of blood sugar utilized. With a fall in the R.Q., to an average of 0.879 and a reduction of 44 p.c.

in sugar utilization, it must be concluded that substances other than carbohydrates have been called upon to supply the energy requirements of the cardiac muscle. In the third hour blood sugar is practically depleted and glycogen continues to be stored, from which it is clear that there is a sparing of the carbohydrate supplies of the heart effected by the synthetic action of insulin. When available blood sugar has been reduced to an almost negligible quantity by insulin and the R.Q. has fallen to 0.760, the heart still endeavours to maintain an approximately normal oxygen consumption with protein and fat forming the great part of the fuel. And, further, from the fact that glycogen is synthesized it must be concluded that the synthetic action of insulin has far outweighed its action as a stimulant of oxidative processes: in fact the synthetic action has dominated the picture throughout, be it in hyperglycæmia or in hypoglycæmia.

SUMMARY.

A method for the more accurate estimation of the R.Q. of the isolated heart has been described.

The gaseous metabolism, the sugar utilization, and glycogen synthesis of the normal heart have been investigated, with a maintained normal blood sugar, in hyperglycæmia and hypoglycæmia, with and without insulin.

Under maintained normal conditions of blood-pressure and blood sugar, the R.Q. is unity, the oxygen consumption is 3.960 c.c. per g. of heart muscle per hour, blood-sugar utilization being equivalent to the oxygen consumed.

Hyperglycæmia is always associated with an increase in oxygen consumption, sugar utilization, and glycogen synthesis.

The effect of hypoglycæmia *per se* on the normal heart has been investigated and it has been shown that, despite a definite depression in the general metabolic activity of the heart, and a progressive loss of glycogen, carbohydrates remain the only source of energy as long as carbohydrate is readily available.

The effect of insulin upon the metabolism of the heart in hyper- and hypoglycæmia has been studied, and it has been shown that, while in hyperglycæmia insulin increases oxygen consumption and sugar utilization to a very small extent, it markedly increases the synthesis of glycogen in heart muscle.

In the presence of a definite hypoglycæmia produced by insulin, the heart, with a R.Q. of unity, shows little increase in oxygen consumption,

but a marked increase in glycogen synthesis. With a progressive and marked reduction in the blood sugar the R.Q. of the heart steadily falls, indicating a progressive increase in the use of protein and fat as sources of energy, sugar utilization diminishes rapidly while glycogen synthesis is maintained. With a continued administration of insulin, the percentage relation between oxidation and synthesis tends to be changed towards the hyperglycæmic insulin ratio, namely from 97 : 3 to 86 : 14.

It has been demonstrated, in so far as the carbohydrate metabolism of cardiac muscle is concerned, that the essentially characteristic action of insulin is that of a stimulant of synthetic processes and not of oxidative metabolism.

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THE EXCRETION OF PROTEIN BY THE MAMMALIAN KIDNEY.

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It has been frequently stated in the literature of the last hundred years that certain proteins are excreted by the healthy mammalian kidney when they are introduced into the blood stream. The purpose of the present research was to investigate this property of the kidney, which was puzzling when considered according to current ideas of renal function. The excretion of gelatin, Bence-Jones protein, egg albumin, hæmoglobin, serum proteins of various animals, edestin, caseinogen and hæmocyanin was studied. Experiments were made both on anaesthetized animals and on isolated perfused kidneys. Histological examinations of the kidneys were made in a certain proportion of the cases.

TECHNIQUE.

Cats and a few rabbits were anaesthetized with amytal, urethane or dial. The urine was collected by means of urethral cannulæ, the bladder being emptied and tied off above the level of entry of the ureters. Injections were made into an external jugular vein, and the blood-pressure was recorded from a carotid artery. The urine obtained from the bladder was always tested for protein at the beginning of the experiment.

Dogs were used for the isolated perfused kidney experiments, which had the advantage that possible complications arising from the influence of the added proteins on the general circulation were avoided. Pump-lung-kidney preparations were employed, similar to those used by Bayliss and Lundsgaard [1932], but with two modifications.

(1) In the pump, the oil no longer comes into direct contact with the blood, but is in contact with a saline solution contained in a rubber finger-stall which projects into the valve chest. Consequently, while the piston continues to be automatically lubricated, there is no risk of any oil contaminating the blood, and there is no blood side-tracked in the pump. If, by any chance, the finger-stall should split during an experiment, the pump reverts to its original condition and the experiment need not be stopped.

(2) In order to avoid any risk of contaminating the blood from the outside of the lungs and heart (by pericardial fat, etc.), a cannula was tied into the auricular-ventricular groove, and the blood issuing from the pulmonary veins led off to the reservoir.

These modifications were made after histological examination of the earlier experiments, which showed fat or paraffin emboli in the kidneys. Bacterial emboli were also seen to be present in some of the early experiments, and the whole apparatus was consequently washed out with alcohol, followed by sterile saline immediately before use. This procedure was successful in eliminating them. The apparatus was in duplicate, and so arranged that two kidneys (ordinarily from the same animal) could be perfused either from a common source of blood, or from two entirely different circuits.

Protein in the urine and in the solutions added to the circulating blood, was estimated by the method of Kerridge [1931]. Care was taken that the quantity of protein added was never less than that necessary to make such a concentration in the blood that, if it were excreted in this concentration in the urine, it would be easily estimated by this method (about 25 mg. protein nitrogen per 100 c.c.). Smaller concentrations (5–25 mg./100 c.c.) were easily detectable. In the anæsthetized animals the protein, dissolved in 0.9 p.c. NaCl, was slowly added through the cannula in the jugular vein. In the perfusion experiments the protein in saline solution was either added initially to the circulating blood, or at a given time in the course of the experiment after a control period. Particulars concerning individual proteins are given together with the results.

The *Creatinine* excretion was followed in most experiments with isolated kidneys, creatinine being added to the circulating blood to raise the concentration to about 10 mg./100 c.c. Estimations were made by the method of Folin as described by Rehberg [1926]. From the concentrations of creatinine in the urine and the serum, we have calculated the glomerular filtration in the manner described by Rehberg [1926]. We are not, however, prepared at this stage to affirm that this figure represents the actual rate of passage of water through the glomerular membranes of the isolated kidney, but we have found it a useful index of the activity of the preparation.

As a rule, a sufficient rate of urine flow was obtained without the addition of any diuretic. In some experiments, both on the intact animal and the isolated kidney, it was necessary to add 25–50 c.c. of normal saline. In no case was urea or glucose added. Toxic reactions (marked reduction in urine flow, blood flow and filtration rate) were obtained when certain of the protein solutions were added to the pump-lung-kidney preparation. These, however, were in most cases less than those obtained when similar quantities of fresh defibrinated blood were added.

Histological examinations were made of a certain number of dogs' kidneys, both experimental and control. The latter included some from dogs used merely as "bleeders."

Thin wedges of the kidneys were fixed in 4 p.c. Schering formaldehyde in 0.9 p.c. NaCl, and sections were prepared from paraffin and frozen blocks. The paraffin sections were stained with Ehrlich's hæmatoxylin and eosin, Weigert's iron hæmatoxylin and van Gieson's mixture, and Weigert's fuchselin and neutral red. Sections cut on the freezing microtome were stained for fat with Scharlach R, and counterstained with Ehrlich's hæmatoxylin. In a few examples portions of kidney were also fixed in Zenker's fluid, but as no additional information was obtained by this procedure the use of this fixative was not continued.

CONTROL OBSERVATIONS.

The first few samples of urine collected from the anæsthetized cats not infrequently contained protein. This very rapidly cleared up, and was probably due to the excitement during the anæsthesia, and possibly, also, to reflexes from the bladder and urethra during the cannulation.

The urine secreted by the isolated kidneys was free from protein, with the exception of the first few samples collected after transference to the pump-lung-kidney circuit. The first 2 c.c. of urine secreted were rejected as being contaminated by urine secreted before excision, and the second 2 c.c. sample contained usually about 20–100 mg. protein nitrogen per 100 c.c., while the protein excretion rate varied from kidney to kidney from 50 to 200 γ of protein nitrogen per min. This proteinuria usually cleared up almost completely in about $\frac{3}{4}$ hour.

The isolated kidneys usually continued to secrete for some 3 hours after the beginning of perfusion. Towards the end of this period both urine flow and filtration rate was small, probably on account of the approaching death of the kidney, but it is to be noted that in no case was there a concomitant increase in protein excretion. Death of the kidney, therefore, is not accompanied by proteinuria.

The time elapsing between clamping the renal artery preparatory to excision of the kidney and the establishment of the flow of blood from the pump was usually between 1 and 2 min. Control experiments have shown that stopping the perfusion pump during the course of an experiment for as long as 5 min. does not give rise to proteinuria, although anæmia for 30 min. does. There was, moreover, no correlation between the magnitude of the initial protein excretion rate, and the time taken in transferring the kidney. We are of the opinion, therefore, that the initial proteinuria is not due to the anæmia produced by the transfer of the kidney from the whole animal to the pump-lung-kidney circuit, but results, in all probability, from the disturbance to the renal nerves incidental to the operation. We are supported in this conclusion by the observations of Livingston and Waggoner [1925], who found that in

the anæsthetized rabbit, obstruction of the flow of blood through the kidney did not produce proteinuria, if care were taken that the renal nerves were not interfered with in any way.

Histological examination of nine kidneys of dogs used merely as "bleeders" was made, of which five could be regarded as normal kidneys. In these the arrangement and appearance of the renal elements resembled in general those found in the human kidney. In particular the glomeruli presented a marked structural similarity. Sclerotic glomeruli, such as are found in all human kidneys in varying extent in the later decades of life, are, however, not present in the examples in this series. Likewise, the different forms of vascular degeneration that are responsible for these appearances in human kidneys are not present in the dog kidneys. In all examples the capsular space in a few of the glomeruli contained a small amount of a vacuolated hyaline eosinophil material of albuminous appearance.

The cytological differences that exist between the first and second convoluted tubules in the human kidney are not present in the dog's kidney. A further difference lies in the prevalence of large quantities of isotropic fatty material in the high cubical epithelium of Henle's loops. This has been observed so constantly both in this and in the two following series that it must be regarded as a normal feature for the mongrel dogs which were used for these experiments.

In one kidney of this series there was a chronic ascending nephritis which, from the post-mortem examination, appeared to be secondary to a myoadenomatous enlargement of the prostate gland. In another kidney a very few of the glomeruli showed a focal necrosis of the tuft, a lesion that is commonly found in human Bright's disease. In this change there is a focal degeneration of a fatty or lipoid nature with loss of the nuclei in the affected portion of the tuft. In the same kidney there was one small focus of periglomerular infiltration with small lymphocytes. In two other examples in this series, also, were found a few small foci of lymphocytic infiltration and, in some of these areas, there was also a little fibrosis. The precise nature of these changes must remain uncertain, but, inasmuch as in two of these dogs the renal lesion was associated with a granulomatous inflammation of the spleen resembling Hodgkin's lymphogranuloma, it may be inferred that the slight chronic focal inflammation of the kidney was part of the same condition.

Three kidneys were examined which had been used as controls in the isolated perfusion experiments, and the alterations in the histological appearances in the dog's kidneys resulting from perfusion for 3-4 hours

are remarkably slight. The only demonstrable change is a certain amount of cedema of the interstitial tissue. This is most conspicuous about the glomeruli and results in the production of a narrow empty halo immediately around Bowman's capsule (Plate I, fig. 1). The glomerular tufts are plump from distension of their capillaries. There is no indication, from the histological appearances of the cells of either glomeruli or tubules, that the nutrition of the kidney has been impaired by perfusion.

In the earlier specimens there were two kinds of artefact produced in the process of perfusion. In the first, observed in sections stained for fat, a number of the glomerular capillaries contained small fat emboli. In the second, which involved both glomerular capillaries and some of the larger vessels, such as the interlobular arteries, the lumina sometimes contained closely packed masses of cocci without any surrounding cellular reaction. The modifications in the technique of perfusion previously referred to stopped the occurrence of these two kinds of emboli, and in the later specimens they were absent or present to only a very trivial extent.

In one example a small focus of lymphocytic infiltration was present in the interstitial tissue of the cortex.

RESULTS.

The results on the anæsthetized animals, and on the isolated perfused organs, agreed with one another and are summarized in the following table, together with the values of the molecular weights of the proteins obtained from the literature.

	Molecular weight of protein		
	By ultra-filtration method*	By ultra-centrifugation method†	By osmotic pressure method‡§
<i>Proteins excreted:</i>			
Gelatin	123,600	35,000	{ >16,500 <66,700 ‡
Bence-Jones	24,500	35,000	—
Egg albumin	33,400	34,500	—
Hæmoglobin	66,800	68,000	67,000§
<i>Proteins not excreted:</i>			
Hæmoglobin	66,800	68,000	67,000§
Serum albumin	45,000	67,500	{ 72,000¶ 75,000
Serum globulin	81,000	103,800	{ >154,000¶ <192,000¶
Edestin	116,000	208,000	—
Casein	192,000	188,000	—
Hæmocyanin (<i>Helix</i>)	—	5,000,000	—

* Cohn [1925].

† Svedberg and co-workers [1930, etc.].

‡ Frankel [1931].

§ Adair [1928].

|| Burk [1931].

¶ Adair and Robinson [1930].

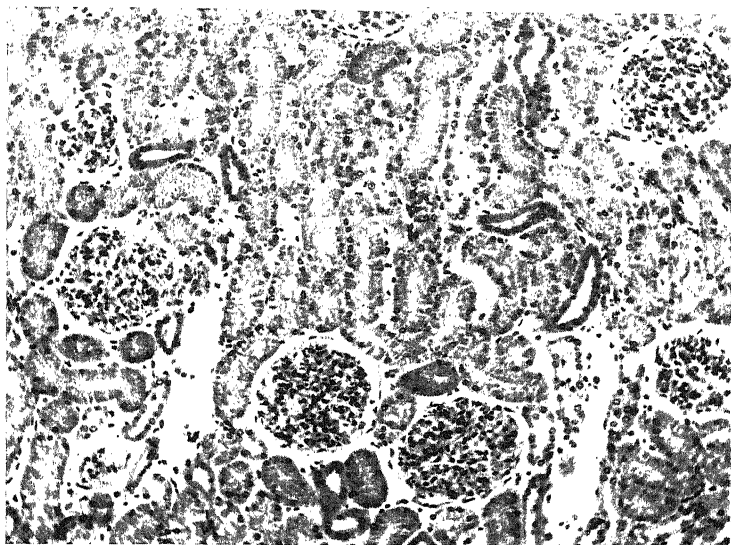


Fig. 1. Section through a dog's kidney which had been isolated and perfused for $1\frac{3}{4}$ hours with normal defibrinated blood. Stained with hæmatoxylin and eosin. Magnification $\times 140$.

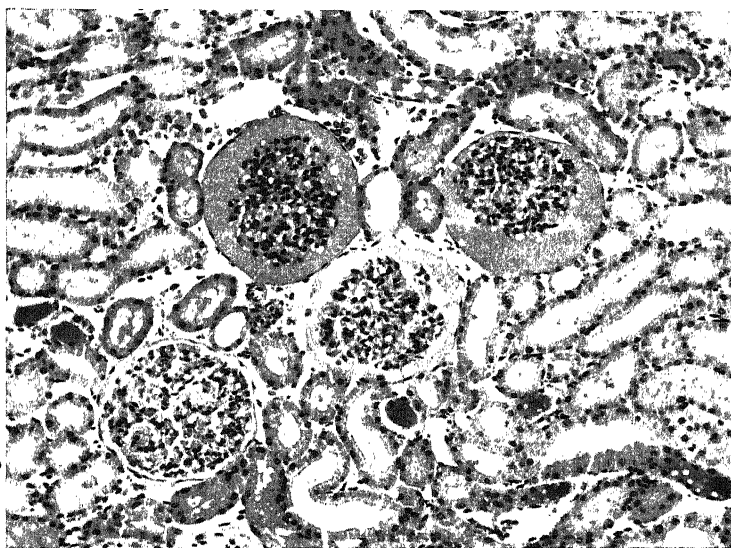


Fig. 2. Section through a dog's kidney which had been isolated and perfused with normal dog's blood for $1\frac{3}{4}$ hours and with blood containing egg albumin at a concentration of approximately 50 mg. protein nitrogen per 100 c.c. blood for $\frac{1}{2}$ hour. Magnification $\times 140$.

Fig. 3 illustrates an experiment on the perfused dog's kidney in which dog's blood containing gelatin was alternately substituted for normal dog's blood without stopping the circulation. In Fig. 4 are plotted the results of an experiment in which egg albumin was added to the blood perfusing a dog's kidney. Fig. 5 is drawn from data obtained from two anæsthetized cats, showing that Bence-Jones protein was excreted, while hæmocyanin was not. The diuretic effect seen in both cases is explained by the 0.9 p.c. NaCl solution in which the protein was dissolved.

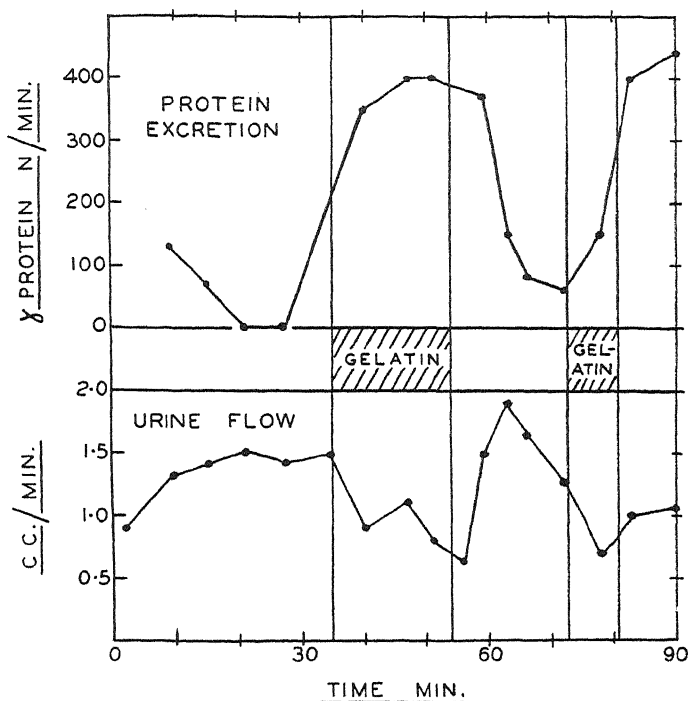


Fig. 3. Pump-lung-kidney preparation. During the period shown, the kidney was perfused with blood containing gelatin (approximately 150 mg. protein nitrogen per 100 c.c.). The resulting proteinuria is seen to be reversible.

Details of the preparation and excretion of the individual proteins are given below.

(1) *Gelatin*. Pickford and Verney¹ have found that gelatin can be excreted by the heart-lung-kidney preparation. Its molecular weight is a little doubtful. The minimum molecular weight, calculated from

¹ Personal communication.

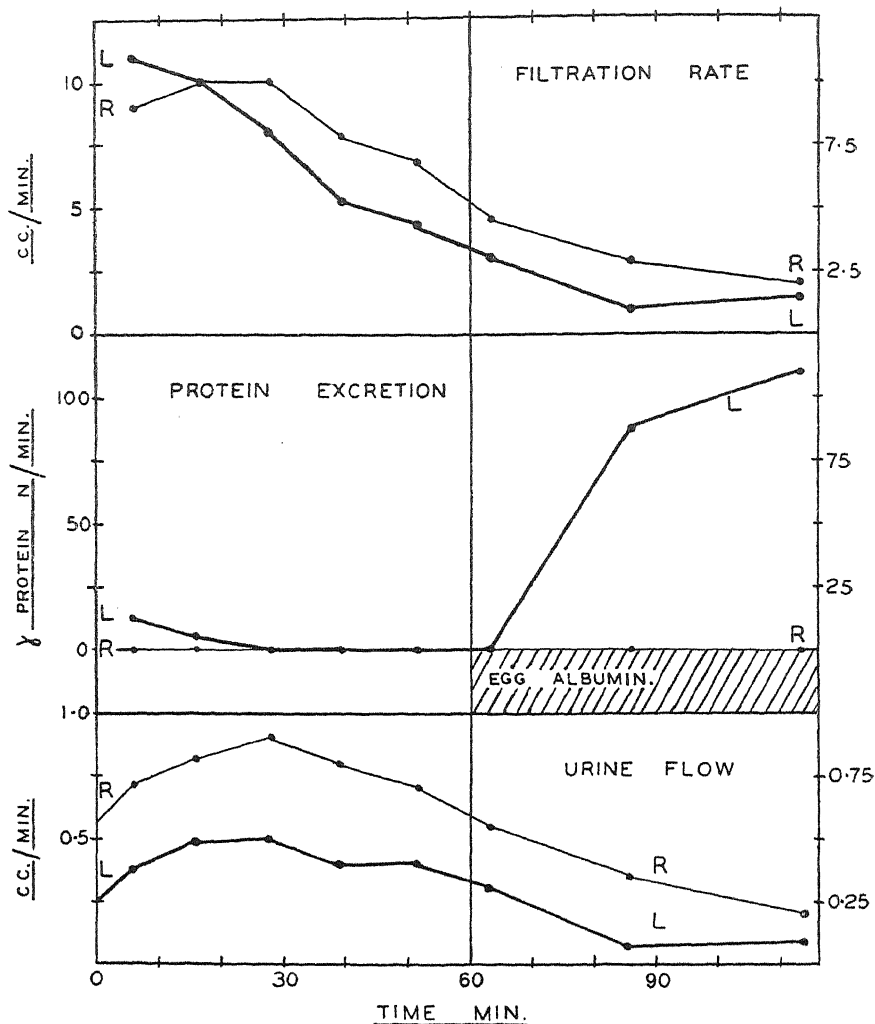


Fig. 4. Pump-lung-kidney preparation. The right kidney was perfused with normal defibrinated blood throughout (thin lines). Egg albumin was added to the blood perfusing the left kidney to make a concentration of about 150 mg. protein nitrogen per 100 c.c. at the moment indicated (thick lines). The filtration rate was calculated from the creatinine excretion.

chemical analyses, is about 10,300 [Cohn, Hendry and Prentiss, 1925], and Krishnamurti and Svedberg [1930], approaching the problem by the method of ultra centrifugation, find that ordinary gelatin solutions are heterogeneous at all H-ion concentrations. At a pH of 7.5 the greater part of the gelatin appears to have a molecular weight of 35,000, but in the vicinity of the iso-electric point (pH 4-6), aggregation takes place, and

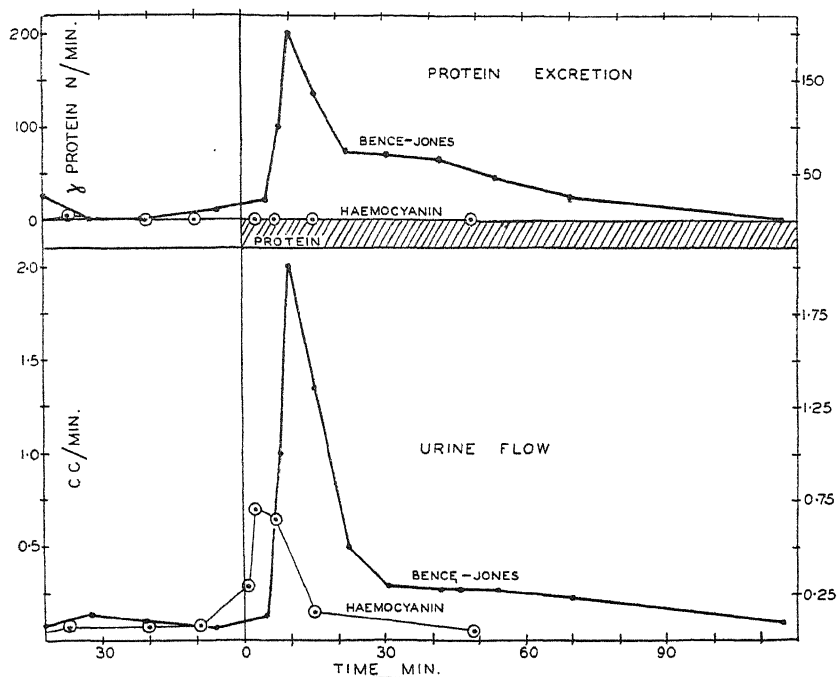


Fig. 5. Composite figure drawn from results obtained on two cats under dial anaesthesia. Into one was injected a solution of Bence-Jones protein containing about 10 mg. protein nitrogen, and into the other a solution of haemocyanin (*Helix*) containing 160 mg. protein nitrogen. The times of injection are made to correspond in the two experiments. The Bence-Jones protein was excreted, the haemocyanin was not.

different preparations may have different molecular weights ranging from 75,000 to 100,000. At all H-ion concentrations, components with a molecular weight of 11,000 were present in addition: Krishnamurti and Svedberg suggest that these may be breakdown products of the true gelatin molecules whose molecular weight is 35,000. Cohn [1925], from a study of the diffusibility of gelatin through collodion membranes, comes to the conclusion that the molecular weight is not less than

123,600. He does not state the H-ion concentration of his solutions, but if, as is very probable, he worked at the iso-electric point, his results do not conflict with those of Krishnamurti and Svedberg. In the circulating blood it seems highly probable that gelatin has a molecular weight of 35,000, there being present, also, a certain proportion of breakdown products with a molecular weight of about 11,000. Frankel [1931], from measurements on the osmotic pressure of solutions of pure commercial gelatin in water, finds that the molecular weight lies between 16,500 and 66,700, the value falling with a rise in temperature (6–38° C.) and rising after removal of traces of salts by prolonged dialysis.

No estimate can be given of the concentrating power of the kidney for gelatin, since this protein is not precipitated by tri-chloroacetic acid, and the method of Kerridge cannot be used for its estimation; we used the quantity of precipitate produced by a standard amount of saturated picric acid as a rough estimate of the protein concentration of our urine samples. The fact that gelatin is not precipitated by trichloroacetic acid, however, enabled us to perceive that there was no simultaneous excretion of serum albumin accompanying the gelatin, and thus making it very improbable that the protein excretion was damaging the kidney.

It was observed, incidentally, that the addition of gelatin to dog's blood results in a very marked agglutination of the corpuscles, and an increased resistance to the flow of blood through the kidney; we cannot say whether this indicates a real vaso-constriction, or is due merely to the agglutination.

(2) *Bence-Jones protein*. Taylor, Miller and Sweet [1916, 1917] have observed that this protein can be excreted in the urine by a normal dog. We have confirmed this observation, and found, in addition, that it is concentrated by the kidney, the concentration ratio being probably not much less than that of creatinine, although our protein preparations were not pure enough for the exact figure to be determined. We are indebted to Dr L. J. Hewitt for our samples which were prepared from the urine of patients at the London Hospital.

(3) *Egg albumin*. We made no attempt to prepare a pure sample of egg albumin, but merely diluted the white of an egg with about 50 c.c. of normal saline solution and filtered it through muslin. It is stated by Claude Bernard [1859] that Berzelius noticed that egg albumin could be excreted by the kidney, and this observation has since been repeatedly confirmed. Our results are a further confirmation, and we have found that the urine contains the protein in a higher concentration than does the circulating blood, the concentration ratio being approximately the same as that of creatinine.

(4) *Hæmoglobin*. It is well known that hæmoglobin can be excreted by the kidney, but it was observed by Pearce, Austin and Eisenbrey [1912] that hæmoglobin is only excreted by the intact dog when its concentration in the blood exceeds a certain threshold value. We have been able to confirm this, and have therefore included it in both sections of the table. On the isolated kidney, the threshold lies between 0.2 and 0.5 g. Hb per 100 c.c. serum. Even when this threshold is exceeded, however, hæmoglobin is not very readily excreted, the concentration ratio being always very much less than that of creatinine, although running parallel to it, and is rarely greater than 1. The threshold value given by Pearce, Austin and Eisenbrey is 0.06 g. hæmoglobin per kg. body weight. Assuming $\frac{1}{25}$ th of the body weight to be plasma, this corresponds to a concentration of 0.15 g. hæmoglobin per 100 c.c. plasma, which is of the same order as the figure obtained by us.

(5) *The serum proteins*. The normal kidney appears never to excrete either serum albumin or serum globulin, even though they be derived from a different species. Thus we have shown that dogs' isolated kidneys do not excrete human, cat or ox serum proteins, and that the kidneys of anæsthetized cats do not excrete human or dog serum proteins. In conformity with the fact that serum albumin has a lower molecular weight than serum globulin, it is well known that in nephritis the urinary proteins contain more albumin than globulin. Thus it appears that even damaged kidneys excrete the smaller molecules more readily than the larger.

In one experiment we perfused two kidneys with a mixture of about 30 p.c. ox blood to 70 p.c. dogs' blood. The ox blood was somewhat hæmolyzed, and hæmoglobin appeared in the urine. The serum proteins of the ox were not excreted at all, even though their concentration in the blood plasma was about the same as that of the hæmoglobin. We are therefore convinced that the same kidney can excrete hæmoglobin, and hold back "foreign" serum proteins. This conclusion is supported by the observations of Cramer [1908], who states that ox serum proteins injected intraperitoneally into normal rabbits are not excreted in the urine, whereas egg albumin, similarly injected, is excreted.

The evidence concerning the relative molecular weights of hæmoglobin and serum albumin is conflicting. Cohn [1925] (ultrafiltration method) considers hæmoglobin to be the larger, Svedberg [1930] (ultracentrifugation method) considers them to be the same, while Adair and Robinson [1930] and Burk [1931] (osmotic pressure measurements) consider hæmoglobin to be the smaller.

(6) *Edestin*. We do not feel much confidence in the results obtained with this protein, owing to its low solubility. It was necessary to dissolve the requisite quantity of the protein (obtained from the British Drug Houses, Ltd.) in 4 p.c. sodium chloride at a pH of 8-9, and to add it to the blood very slowly, so that it did not precipitate out. Moreover, Svedberg and Stamm [1929] have shown that in concentrations corresponding to saturation in the blood serum, edestin is unstable, and tends to break down into products of low molecular weight, not identifiable by the ultracentrifuge. In conformity with this, we observed a small proteinuria in one cat, after the intravenous injection of 10 c.c. of a 2.4 p.c. solution of edestin in 4 p.c. NaCl at a pH of 8.5.

(7) *Casein*. We used "Light White Casein" from the British Drug Houses, Ltd. The molecular weight of casein appears to depend upon the manner in which it is prepared. Thus, Svedberg, Carpenter and Carpenter [1930] obtained (1) a preparation consisting of a mixture of substances with molecular weights ranging from 75,000 to 100,000, (2) a preparation consisting almost entirely of a substance with a molecular weight of 188,000, with a small admixture of a substance with a molecular weight of 375,000, and (3) a preparation containing only the substance of molecular weight 375,000. While it seems fairly probable that in the circulating blood most of the casein would have a molecular weight of 188,000, the matter is not of great significance, since the lowest molecular weight given is greater than 68,000, which appears to be the excretion limit for the normal kidney.

(8) *Hæmocyanin*. No attempt was made to obtain a pure preparation of hæmocyanin. About thirty *Helix pomatia*¹ were bled by careful incision of the heart, the mixed blood centrifuged to remove sediment, and dialysed in the cold for 6 days against 0.9 p.c. NaCl. The final solution contained 450 mg. protein nitrogen per 100 c.c.

Histological preparations were made from eight kidneys which were excreting protein, and in general the appearances could not be distinguished from those of the control perfused kidneys. In one kidney, however, which was excreting egg albumin in high concentration at the time of fixation, there is a conspicuous ballooning of most of the glomerular capsules with a dense homogeneous hyaline eosinophil substance (Plate I, Fig. 2). This substance is denser and more eosinophil than the scantier material found in the capsular spaces in the control kidneys. The lumina of the tubules are also filled with similar material. In another

¹ We are indebted to Mr N. H. Howes for these snails and for his assistance in preparing the hæmocyanin solutions.

kidney, which was also excreting egg albumin, but in a lower concentration, the amount of "albuminous" material in the capsules is in excess of that usually present.

CONCLUSIONS.

Our results show that while the mammalian kidney can excrete some proteins, it cannot excrete others. The distinction does not appear to depend on chemical structure, or biological origin, but on molecular weight. Proteins with molecular weights less than about 70,000 are excreted, those with heavier molecules being retained. The histological preparations showed no evidence of damage in kidneys which were excreting protein. In two experiments, in which large amounts of egg albumin were being excreted, there was positive evidence that the glomeruli were the sites of excretion. This is in accordance with the observations of several workers of 40-50 years ago (for references, see Cushny, 1926).

The filtration theory of glomerular function has postulated that the glomerular membranes are impermeable to all proteins. Cushny [1926] states that "the excretion of injected protein seems to occur only with injury to the capsule." Our chemical and histological evidence does not support either of these postulates, but the observed phenomena can all be easily accounted for by the filtration theory, if it be assumed that the glomerular membrane is permeable not only to crystalloids but also to proteins of molecular weight less than 70,000. Indeed, it is difficult to imagine any other theory which would explain so easily the differentiation of proteins by the kidney according to their molecular weights.

SUMMARY.

The excretion of proteins by anæsthetized cats and rabbits and by the isolated and perfused kidneys of dogs has been studied.

Gelatin, Bence-Jones protein, and egg albumin were excreted and concentrated; serum albumin and globulin of their own and other species, edestin, casein and hæmocyanin were not excreted. Hæmoglobin was excreted only when its plasma concentration exceeded a certain level, and was little concentrated.

The proteins which were excreted all have molecular weights less than 70,000, while those which were not excreted all have molecular weights greater than 70,000. Hæmoglobin has a molecular weight of 68,000 and is thus near the border-line.

Histological examination showed no evidence of renal damage and protein was seen in the capsular space in two experiments in which egg albumin was excreted in high concentration.

An account has been given of the histological picture of the normal dog kidney, and of the kidney which has been isolated and perfused for 3-4 hours; the difference between them is surprisingly small.

Our results are explained by the filtration theory of glomerular function if it be assumed that the glomerular membrane is permeable not only to crystalloids but also to proteins of a molecular weight less than 70,000.

It is our pleasure to thank Prof. Arthur Ellis and Prof. C. Lovatt Evans for advice and criticism.

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THE PORTAL CIRCULATION.

II. The action of acetylcholine.

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IN a previous paper [McMichael, 1932] it was shown that adrenaline constricted the portal venous ramifications within the liver, thus confirming the views of others, *e.g.* François-Franck and Hallion [1896], and Griffith and Emery [1930], that the post-ganglionic splanchnic nerves passing into the liver carry constrictor fibres to the portal venules. In addition, it was shown that when the splanchnic arterioles dilated with the fall in arterial pressure after adrenaline injection, there occurred a secondary rise in portal pressure, due to increased inflow into the portal system. Since recent work by Pick [1931], and by Grab, Janssen and Rein [1929], has tended to stress the importance of the hepatic vascular bed in regulating the flow of blood to the right heart, it is desirable to know whether there is a parasympathetic supply having the converse effect of adrenaline and the sympathetic nerves on the hepatic vascular system. It was thought that acetylcholine might yield a clue to this problem, and the series of experiments detailed in this paper was therefore carried out, as a corollary to the work with adrenaline.

TECHNIQUE AND METHODS.

The arrangement of the experiments was in most respects identical with that used for the adrenaline investigations [McMichael, 1932]. The work was carried out entirely on cats under chloralose anaesthesia, and the dosage of acetylcholine ranged from 0.0005 to 0.5 mg. for an average cat of 3 kg. The dosage and mode of administration of the drug is indicated in each tracing, or its accompanying legend. The portal and vena cava pressures were recorded by water manometers connected to the stump of the splenic vein and the left renal vein respectively. The liver volume was recorded by a plethysmograph after the model devised by Griffith and Emery [1930]. The flow from the inferior mesenteric vein was measured by a Condon drop recorder. To record changes occurring in the stroke

volume of the heart, a Henderson glass cardiometer was used, connected to a tambour with a slack rubber membrane. As it proved difficult and also unnecessary for the present purposes, to take records showing the diastolic heart volume, a side valve was provided on the connecting tube. In this way diastolic volume is not indicated in the cardiometric tracings, which only show the relative magnitude of the rapid change in volume which occurs in systole. In the cat it was found difficult to take more than three records of the type mentioned at any one time, but as the general nature of the changes did not vary, it is possible to piece the observations together into a composite picture, with some degree of certainty as to their value in relation to one another.

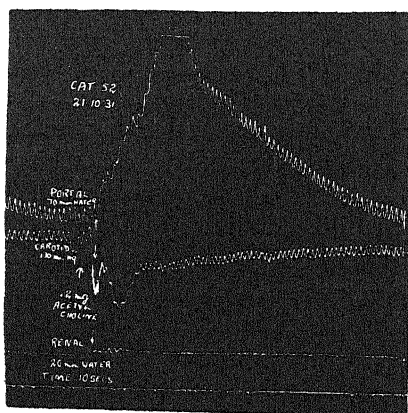


Fig. 1.

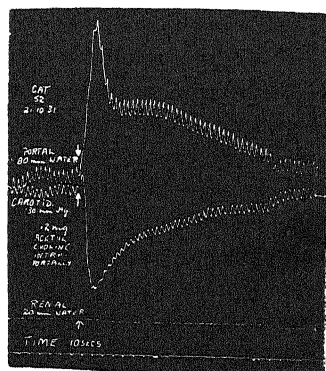


Fig. 2.

Fig. 1. Upper record: portal venous pressure. Middle record: carotid pressure. Lower record: vena cava pressure recorded from renal vein. Time record: 10 sec. At the first arrow 0.2 mg. acetylcholine was injected into the saphenous vein. The second series of three arrows indicates corresponding times about 6 sec. after the arterial pressure has begun to fall. At this time the portal venous pressure begins to rise, while little significant change occurs in the vena cava pressure.

Fig. 2. Upper record: portal venous pressure. Middle record: arterial pressure. Lower record: vena cava pressure from renal vein. Time record: 10 sec. At the time marked by the arrows 0.2 mg. acetylcholine was injected intraportally. There is an immediate rise in portal pressure followed in a few seconds by a fall in arterial pressure. No significant change is seen in vena cava pressure.

I. THE EFFECT OF LARGE DOSES OF ACETYLCHOLINE.

The injection of 0.2–0.5 mg. acetylcholine into a systemic vein produces, as is well known, a marked fall in blood-pressure, together with slowing of the heart. The portal pressure, after showing no change for

some 5 or 6 seconds following the commencement of the drop in arterial pressure, then begins to rise (Fig. 1). This rise in portal pressure is gradual during the first few seconds, and then it climbs more steeply, ultimately falling away as the arterial pressure returns to normal. The injection of a similar dose, or even of a somewhat smaller dose, directly into a mesenteric vein, invariably produces a steep rise in portal pressure, which occurs before any change is seen in the arterial pressure (Figs. 2, 3). It is probable therefore that the rise in portal pressure, caused by the

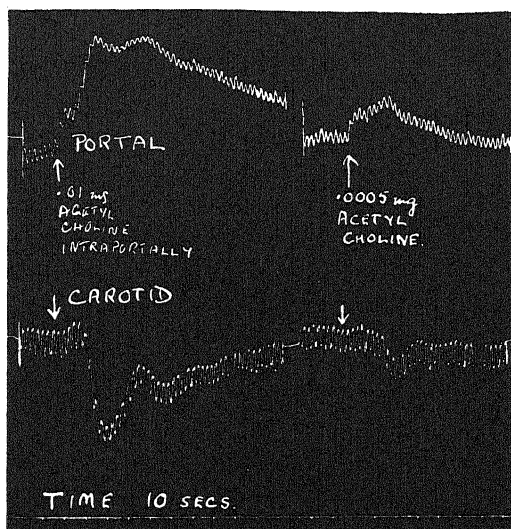


Fig. 3. Upper record: portal pressure. Lower record: carotid pressure. Time record: 10 sec. The intraportal injection of small doses is seen to bring about a rise in portal pressure, which results from a direct constricting effect on the vein.

injection of large doses of acetylcholine into systemic veins, is due to direct action on the portal vein, producing either constriction of the portal venous ramifications in the liver, or diminution in the calibre of the main trunk of the vein itself. Since there is no change in liver volume immediately after intraportal injection of the drug (Fig. 6), it is likely that the action is a general one on the musculature of the portal venous system. A somewhat similar effect was noted by Fleisch [1931], who observed that large doses of acetylcholine caused constriction of the mesenteric veins. Since this action is probably exerted directly on plain muscle, and is not related to the parasympatho-mimetic action of the drug, its nature will not concern us further in this investigation.

II. THE EFFECT OF SMALL DOSES OF ACETYLCHOLINE.

(a) *The portal pressure changes.*

Doses of acetylcholine under one-fifth of a milligramme injected into a systemic vein produce a succession of changes in the portal pressure different from those described above. The first change takes place in the arterial pressure (Fig. 4). This falls away steeply, while the portal pressure

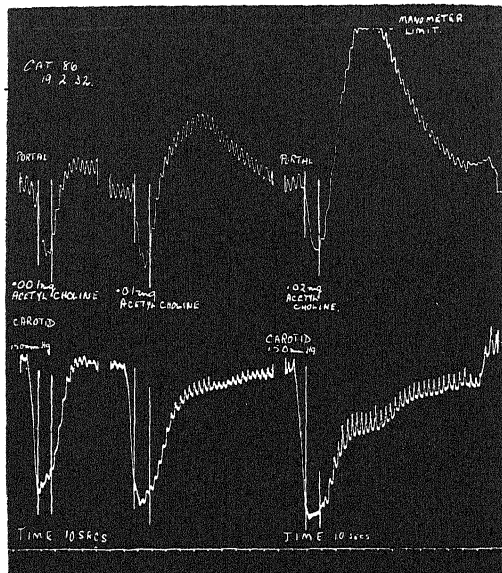


Fig. 4. Upper record: portal pressure. Lower record: arterial pressure. Time record: 10 sec. Three successive records from the same animal showing the effect of different small doses of acetylcholine into the saphenous vein. Vertical lines mark corresponding times. 0.001 mg. brings about a fall in portal pressure with a scarcely significant secondary rise. With increasing doses (0.01 and 0.02 mg.) the secondary rises become more marked.

remains unchanged for some 6 seconds before it also begins to fall. As the cardiac slowing passes off it is followed by an acceleration of the heart beat, above the normal, for a few seconds. About this time the fall in portal pressure ceases, and is replaced by a rise. The extent of this rise is dependent upon the dosage of acetylcholine and the degree of the initial fall in blood-pressure. With a dose of 0.02 mg. the blood-pressure has fallen very considerably and the subsequent rise in portal pressure is

correspondingly well marked. With smaller doses of acetylcholine, the rise in portal pressure becomes less prominent.

Excluding general effects on the calibre of the portal vein as a whole, the cause of a fall in portal pressure may be either increased outflow or diminished inflow. In view of the work of Dale [1914], and the general acceptance of the hypothesis that acetylcholine dilates arterioles, thus causing an increased flow through these vessels into the veins, it was thought that the most probable cause of the initial fall in portal pressure would be increased outflow from the portal venous system. To investigate this possibility records were taken of the effect of these small doses of acetylcholine on the liver volume, and on the pressure in the vena cava.

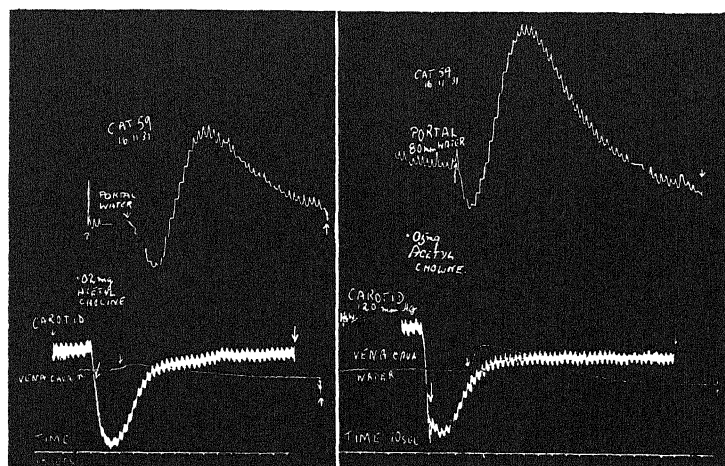


Fig. 5. Upper record: portal pressure. Middle record: carotid pressure. Lower record: vena cava pressure. Time record: 10 sec. Note the abscissae marked by arrows on the right of each tracing. (a) 0.02 mg. acetylcholine into the saphenous vein. The portal pressure begins to fall 5 or 6 sec. after the arterial fall, and simultaneously (arrows) the vena cava pressure undergoes a very slight rise. (b) 0.05 mg. acetylcholine into the saphenous vein. The fall of arterial pressure is accompanied by marked cardiac slowing. Simultaneously (arrows) with the fall in portal pressure a distinct rise in vena cava pressure is seen.

(b) *The effect of acetylcholine on liver volume and vena cava pressure.* (See also Section (g).)

With doses of acetylcholine ranging from 0.001 to 0.2 mg. the effect is constantly a diminution of liver volume (first part of Fig. 15). This diminution in the size of the liver begins about 6 seconds after the arterial

pressure has begun to fall, *i.e.* at the same time as the fall in portal pressure. Fig. 5 shows simultaneous tracings of arterial pressure, portal pressure and vena cava pressure. It is seen that, with smaller doses of acetylcholine (0.02 mg.), little change occurs in the pressure in the vena cava. Larger doses, however, cause a rise in vena cava pressure which occurs at the same time as the fall in portal pressure, and presumably also simultaneously with the diminution in liver volume. This rise in vena

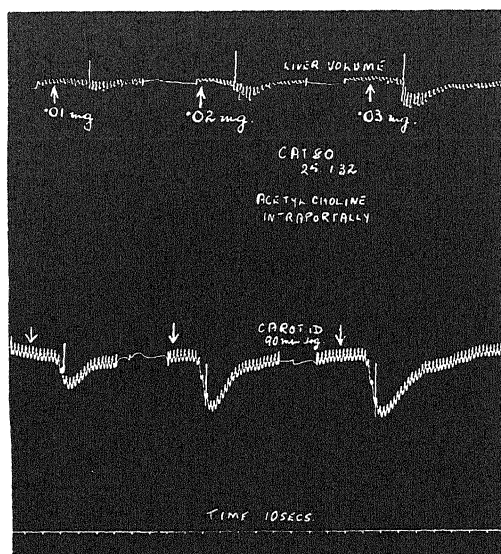


Fig. 6. Upper record: liver volume. Lower record: carotid pressure. Time record: 10 sec. Successive doses of 0.01, 0.02 and 0.03 mg. acetylcholine were injected intraportally. It is seen that no change occurs in liver volume until the fall in arterial pressure appears. The diminution in liver volume is proportional to the degree of the fall in blood-pressure.

cava pressure is only present when cardiac slowing is marked. It was thought at first that this series of changes might be interpreted by assuming that acetylcholine exerted a dilator action on the hepatic veins, thus causing an increased outflow from the liver lobules, with a consequent fall in the liver volume and portal pressure, and an increased inflow into the vena cava. This increased inflow into the vena cava would manifest itself as a rise in vena cava pressure, only in the presence of cardiac slowing: otherwise the heart would be able to deal with the increased delivery of blood quite efficiently, and the vena cava pressure would remain un-

altered. Experiments carried out to test this hypothesis, however, showed that it was incorrect.

The invalidity of the assumption was at once proved by the experiment illustrated in Fig. 6. If acetylcholine dilated the hepatic veins, we should be able to demonstrate a fall in liver volume by the injection of a suitable dose into the portal system, and this diminution in volume should precede any effect on the general systemic circulation. As the illustration shows, this postulate is not fulfilled. No alteration in liver volume occurs until the arterial pressure has fallen, and further, the diminution in liver volume appears to be dependent upon the extent of the fall in blood-pressure.

The true explanation of the initial fall in portal pressure, and of the concomitant diminution of liver volume, is therefore to be sought in the changes which occur in inflow rather than in alterations of the outflow from the portal system.

(c) *The flow of blood through the splanchnic arterioles
under the influence of acetylcholine.*

Fig. 7 shows the type of result constantly obtained when the flow from a mesenteric vein is measured under the influence of acetylcholine. During the first 6 seconds after the arterial pressure begins to fall, there is little change in the rate of flow. A distinct slowing then makes its appearance, and becomes most marked about the turning-point of the arterial pressure curve. As the pressure rises the slowing continues to be pronounced, and as the pressure reaches a level approaching its original height the rate of flow again returns almost to its original rate. Thus the initial fall in portal pressure, which occurs with acetylcholine, appears to be dependent on diminished inflow into the portal system through the splanchnic arterioles.

This result was at first surprising, in view of the opinions generally held as to the action of acetylcholine. It must be remembered, however, that the observations from which conclusions were reached regarding the action of the drug on the arterioles, were not as a rule made on the intact animal, but on isolated perfused organs and tissues. This finding does not therefore in any way invalidate the classical work on the vaso-dilator action of the "vagus substance." It is of interest in passing to note the difficulty which Reid Hunt [1914] found in accepting the work of Dale [1914]. The former writer was unable to detect vaso-dilatation with acetylcholine, and found himself forced to explain the fall in blood-pressure by diminished cardiac output.

Accepting the view, which has been amply proved by other workers, that acetylcholine dilates arterioles, we have two possible explanations for the diminished inflow into the portal system in the intact animal. The first is, that the vaso-dilatation may be more marked in the limb vessels, and the second, that the cardiac output may be diminished during the first stage of acetylcholine action.

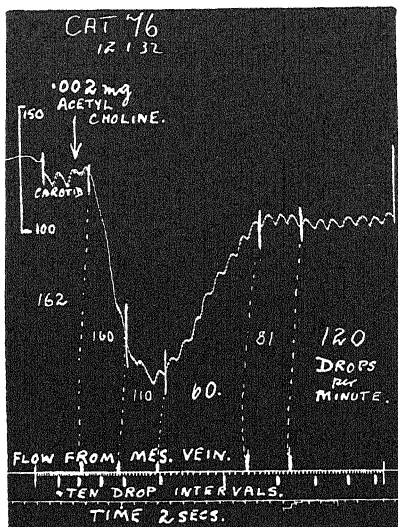


Fig. 7. Upper record: carotid pressure. Lower record: flow from a mesenteric vein in drops. Time record: 2 sec. Average rates are given in each marked interval. At the arrow 0.002 mg. acetylcholine was injected into the saphenous vein. During the first 6 sec. there is little change in the rate of flow. A distinct slowing then makes its appearance. When the arterial pressure begins to rise the slowing becomes still more marked, and the flow only begins to increase again when the blood-pressure approaches the normal level.

(d) *The flow of blood through the limb vessels under acetylcholine.*

Fig. 8 shows the flow of blood from the femoral vein under the influence of acetylcholine. During the stage of cardiac slowing the flow is somewhat diminished. As soon as this stage has passed off, however, a distinct acceleration of flow above the normal level is seen, which gives way to a slowing as the pressure begins to rise again. It appears then that the cardiac output must be diminished during the stage of cardiac slowing, since there is no acceleration of the flow either in the splanchnic area or

in the limbs. The subsequent acceleration which occurs in the flow from the femoral vein indicates, however, the persistence of vaso-dilatation in the limbs until the pulse rate has begun to recover. Even in the intact animal therefore, we find evidence of vaso-dilatation in the limbs, although this is masked by the diminution in cardiac output during the stage of cardiac slowing. In the intact animal there is no such evidence of vaso-dilatation in the splanchnic area, so that if such exists, it must be less marked than that which we have found in the extremities.

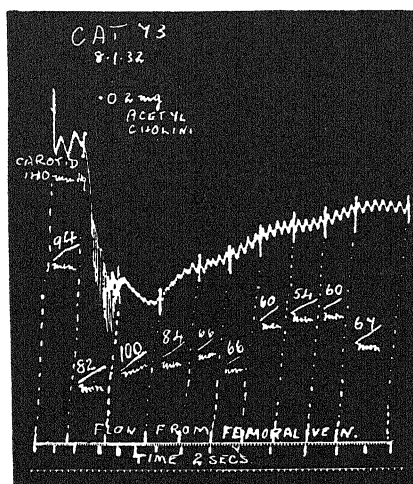


Fig. 8. Upper record: carotid pressure. Lower record: flow from femoral vein in drops. Time record: 2 sec. Following the injection of 0.02 mg. acetylcholine into the jugular vein the blood-pressure falls, and marked cardiac slowing occurs. During the stage of cardiac slowing, there is a diminution of the flow from the femoral vein. When the bradycardia passes off the flow is increased but is later diminished during the recovery of the blood-pressure.

(e) *The cardiac output under acetylcholine.*

The output of the heart under this drug may be influenced in various ways. The slowing of the heart itself may diminish the minute volume, although the stroke volume may be increased. Secondly we have the effect of peripheral vaso-dilatation in increasing the capacity of the vascular system, so that less blood returns to the heart, and its output is diminished.

Regarding the first possibility there can be no reasonable doubt. We have mentioned the evidence of diminished cardiac output in discussing the changes in flow through the limbs during the stage of cardiac slowing. There is also evidence of it in the rise in vena cava pressure, which has

already been noted to occur only when the cardiac slowing is marked (Fig. 5). With the vagi cut to accentuate cardiac slowing from acetyl-

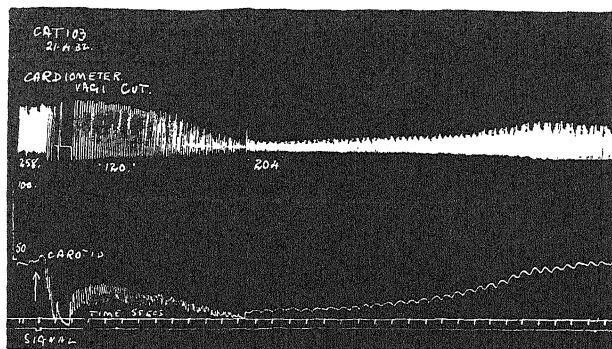


Fig. 9. Upper record: cardiometer. Lower record: carotid pressure. Time record: 5 sec. At the first arrow 0.2 mg. acetylcholine was injected. With the fall in blood-pressure, the heart is slowed from 258 to 120 per min., and the amplitude of each beat is increased. For a few seconds the heart beat becomes irregular, and at this stage the output per beat is markedly diminished. The blood-pressure then begins to recover, and the output per beat gradually returns to normal.

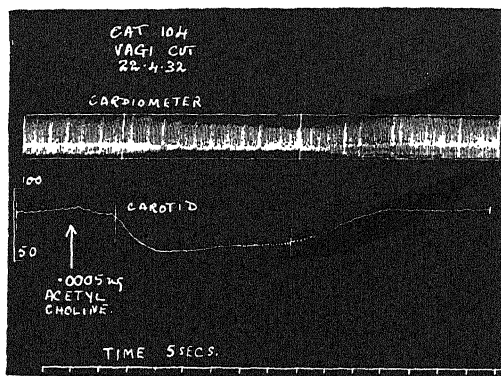


Fig. 10. Upper record: cardiometer. Lower record: carotid pressure. Time record: 5 sec. At the arrow 0.0005 mg. acetylcholine was injected. This dosage is insufficient to produce cardiac slowing. As the blood-pressure falls the cardiac output becomes diminished, and increases again as the pressure rises.

choline, the cardiometer shows an increased output per beat, which, however, is insufficient to maintain the normal minute volume (Fig. 9).

The second factor, *i.e.* increased capacity of the vascular system, is one

which plays a special part in the failing preparation in which there is a condition of oligæmia. The volume of the circulating blood is diminished, and vaso-dilatation in the periphery further diminishes the return of blood to the right heart, with resultant diminution in cardiac output independent of slowing (Fig. 10). In the failing preparation the effect of acetylcholine on the portal circulation is modified, and the fall in portal pressure becomes the predominant feature, while the secondary

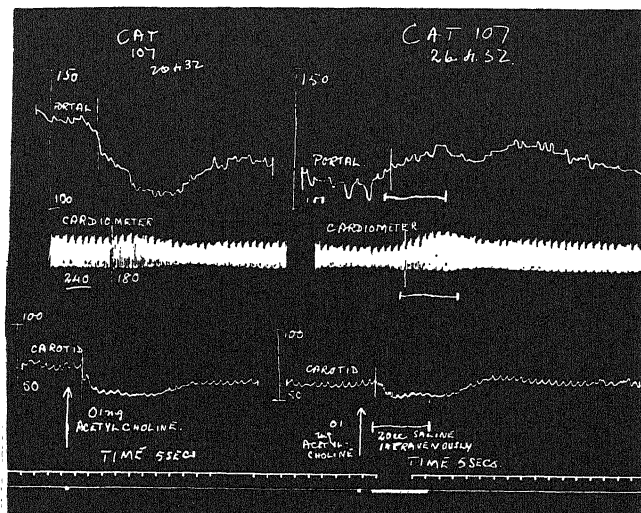


Fig. 11. Upper record: portal venous pressure. Middle record: cardiometer. Lower record: carotid pressure. Time record: 5 sec. At the arrow, 0.01 mg. acetylcholine was injected into the saphenous vein. The heart rate is slowed from 240 to 180, and as the slowing passes off, a diminution in output becomes evident. The fall in portal pressure corresponds to the stage of cardiac slowing with diminished cardiac output. The experiment was repeated with the injection of 20 c.c. saline into the circulation immediately after the acetylcholine injection. The cardiac output was raised with a corresponding rise of portal pressure.

rise is usually absent (Fig. 11). The reason for this will be discussed later (p. 418). Under these conditions the portal pressure fall is seen to follow closely the diminution in cardiac output, and the portal fall is abolished when the cardiac output is artificially maintained.

Thus we see that the fall in portal pressure which occurs after the injection of acetylcholine, is dependent on two factors: a predominance of vaso-dilatation in the limbs deflecting blood away from the splanchnic area, and diminished cardiac output.

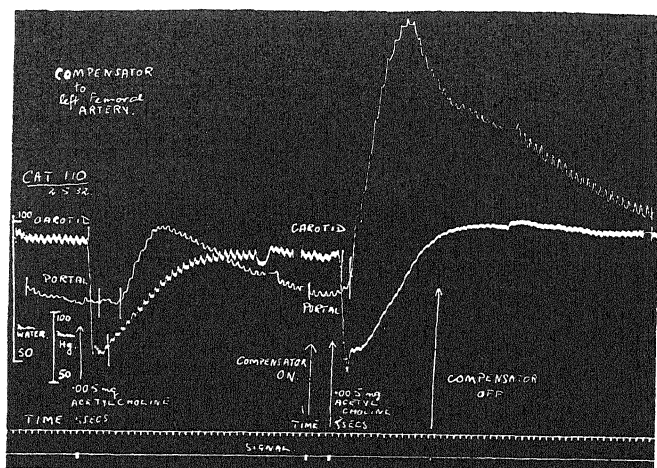


Fig. 12. Upper record: carotid pressure. Lower record: portal pressure. Time record: 5 sec. Bayliss compensator attached to the femoral artery. Acetylcholine (0.005 mg.) was injected into the saphenous vein. The usual fall in portal pressure is not evident, but after a delay a secondary rise is seen. With the compensator in the circuit, a marked rise in portal pressure begins immediately the carotid pressure starts to fall.

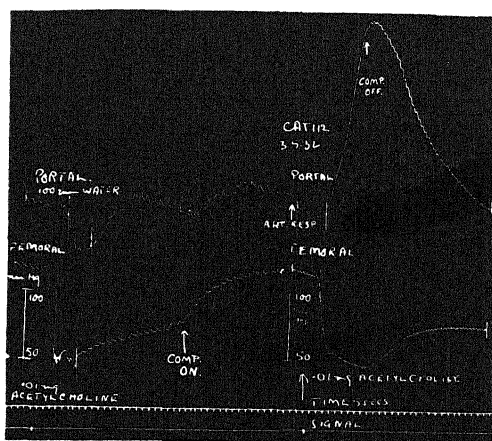


Fig. 13. Upper record: portal venous pressure. Lower record: arterial (femoral) pressure. Time record: 5 sec. Bayliss compensator attached to carotid artery. Following the injection of 0.01 mg. acetylcholine a fall in arterial pressure is accompanied by a fall in portal pressure. With the compensator in communication with the circulation, a rise of portal pressure is seen as soon as the arterial pressure begins to fall, with the same dose of acetylcholine.

(f) The state of the splanchnic arterioles under acetylcholine.

Since we have detected no increase in the flow through the mesenteric arterioles, and since the portal pressure falls and the liver volume diminishes under acetylcholine, the assumption that the splanchnic vessels are dilated would be mere surmise. With a diminished flow through the splanchnic vessels in the presence of diminished cardiac output,

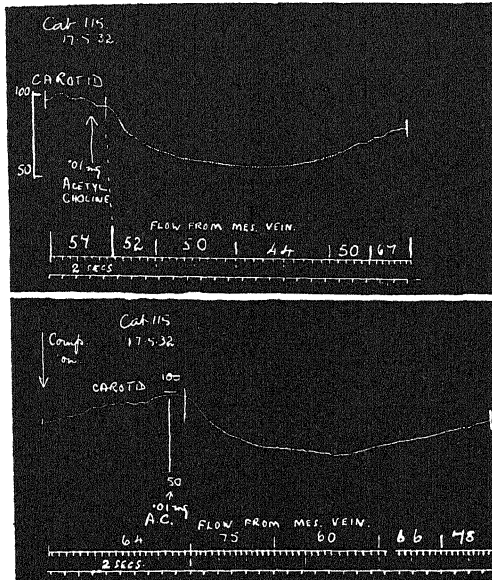


Fig. 14. In both tracings: Upper record: carotid pressure. Lower record: flow from a mesenteric vein (in drops). Time record: 2 sec. Compensator attached to femoral artery. In the upper record 0.01 mg. acetylcholine injected into the saphenous vein brings about a fall in blood pressure with a concomitant diminution of flow through the mesenteric vessels. A few minutes later, with the compensator in communication (lower record), a similar dose of acetylcholine is followed by an immediate acceleration of the flow, with slowing later.

these vessels may be dilated, contracted, or unaltered in calibre. If we could maintain the amount of blood in the arterial system at a constant level, vaso-dilatation in the splanchnic area, if present, should be demonstrable. One way in which it would come to light, would be by an immediate rise in portal pressure as soon as the arterial effect of acetylcholine had manifested itself. These conditions can be achieved by the use of the Bayliss [1908] compensator. Fig. 12 shows the effects of acetyl-

choline under these conditions. There is a prolonged delay, instead of a slight fall, in the first record of portal pressure under acetylcholine, and then the secondary rise is seen as usual. With the compensator in communication with the circulation, the effect is an immediate and pronounced rise in portal pressure at the moment when the arterial pressure begins to fall. The compensator was attached to the femoral artery, and

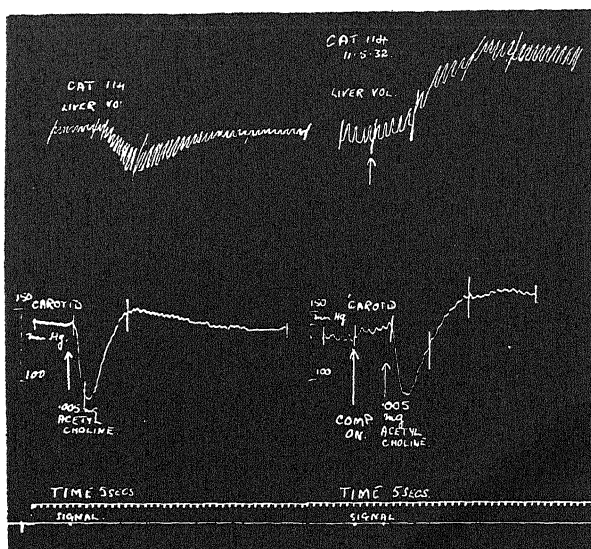


Fig. 15. Upper record: liver volume. Lower record: carotid pressure. Time record: 5 sec. Compensator attached to the femoral artery. 0.005 mg. acetylcholine into the saphenous vein gives a diminution of the liver volume beginning about 6-8 sec. after the fall in arterial pressure. The compensator is then connected to the circulation and, instead of diminishing, the liver increases in volume along with the fall in arterial pressure.

under these conditions tracings may not show the initial fall in portal pressure as one hind limb is out of the circulation, and its function in deflecting blood from the splanchnic area is in abeyance. When, however, the compensator is attached to the carotid artery (Fig. 13), the initial fall in portal pressure is still seen. With the compensator in communication with the circulation, the fall in portal pressure is replaced by an immediate rise, beginning as soon as arteriolar dilatation is evidenced by the drop in arterial pressure.

Fig. 14 shows the effect of compensation on the flow through the mesenteric arterioles. The upper tracing shows the usual slowing of the

flow as already described (Section (c)). With the compensator communicating with the femoral artery, the initial slowing is converted into an increased rate of flow. There is no doubt therefore that the splanchnic arterioles are dilated by acetylcholine, and, when the arterial system is kept filled artificially, that they can transmit an increased amount of blood to the portal system thus causing a rise in portal pressure.

The diminution in liver volume which was described in Section (b) is due to diminution in the flow through the splanchnic arterioles. As in the reversal of the effects of acetylcholine on portal pressure and on flow from the mesenteric veins, the compensator also reverses this action on liver volume. Instead of undergoing a shrinkage the liver is seen to swell (Fig. 15).

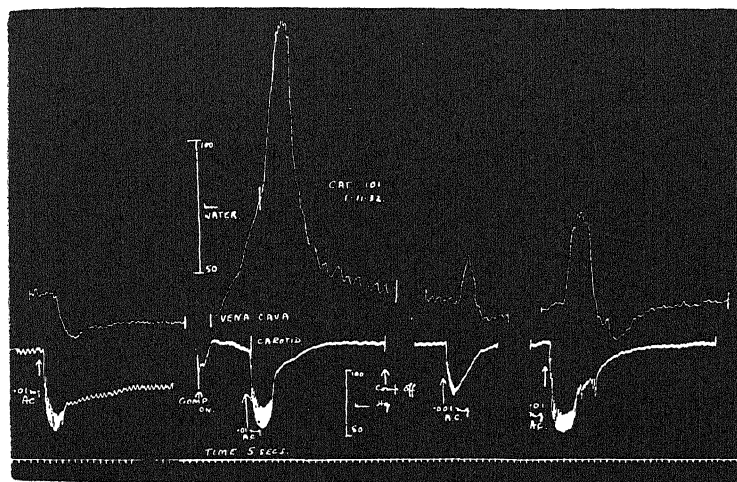


Fig. 16. Upper record: vena cava pressure. Lower record: carotid pressure. Time record: 5 sec. With the injection of 0.01 mg. acetylcholine a fall of vena cava pressure occurs. The arterial pressure does not recover in the usual manner. The compensator is then put into communication with the circulation, and a restoration of blood-pressure with a rise of vena cava pressure results. 0.01 mg. acetylcholine then causes a sudden and steeper rise in vena cava pressure, which ultimately returns to a normal level with the recovery of blood-pressure. The compensator is then excluded from the circulation and different doses of acetylcholine then produce rises in vena cava pressure which only last as long as the cardiac slowing.

(g) General venous pressure effects.

In Section (b), preliminary mention was made of the effects of acetylcholine on vena cava pressure. A rise in vena cava pressure is only seen in the presence of pronounced cardiac slowing. Slowing of the heart alone,

however, is not sufficient to determine a rise in pressure in the vena cava, as can be seen from the first curve in Fig. 16. In this particular preparation, the volume of the circulating blood had to be increased, before the rise in vena cava pressure became evident. Cardiac slowing then determined the extent and duration of the rise. With a slight degree of slowing, the rise of vena cava pressure was slight, and with more marked bradycardia the rise became correspondingly higher and more prolonged.

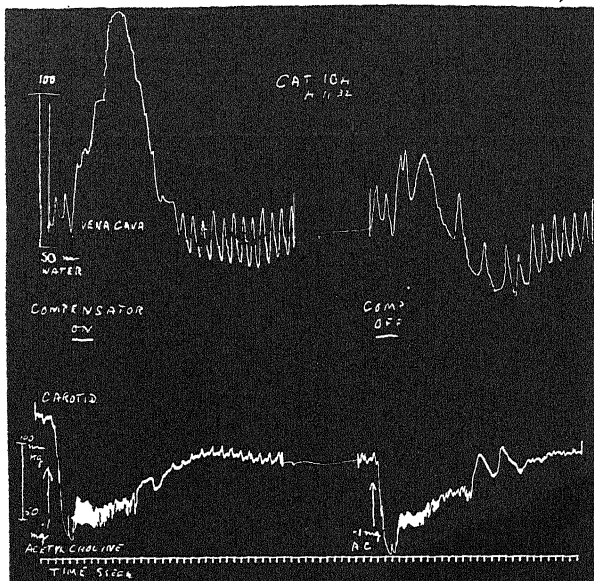


Fig. 17. Upper record: vena cava pressure. Lower record: carotid pressure: Time record: 5 sec. With the compensator in communication with the circulation 0.1 mg. acetylcholine was injected into the saphenous vein. During the stage of cardiac slowing, a pronounced rise of vena cava pressure is seen. When the compensator is excluded from the circulation this rise of vena cava pressure does not occur.

When the blood-pressure falls with acetylcholine, the first effect of the compensator is to pour more fluid into the circulation. If cardiac slowing is present this excess of fluid brings about a rise of vena cava pressure. When the slowing passes off and the heart in consequence becomes more efficient, the excess of fluid is removed from the venous side of the circulation and the venous pressure falls (Fig. 17).

It is unlikely that these general venous pressure effects could take place without some influence on the portal pressure. If we take the initial

portal pressure fall and correlate it with the changes of inflow which occur at the same time, we find that the inflow is reduced on an average (four experiments) from 136 to 99 drops per minute. The volume flow through the portal vein must be diminished in the same proportion. The initial portal pressure fall is about 20 mm. water as a rule, *i.e.* from 80 mm. down to 60 mm. water. By Poiseuille's law, volume flow through narrow vessels

$$V = \frac{\pi r^4}{8\eta} p,$$

where r is the radius of the blood vessel, η the coefficient of viscosity of the fluid (blood), and p the pressure. With small doses of acetylcholine we

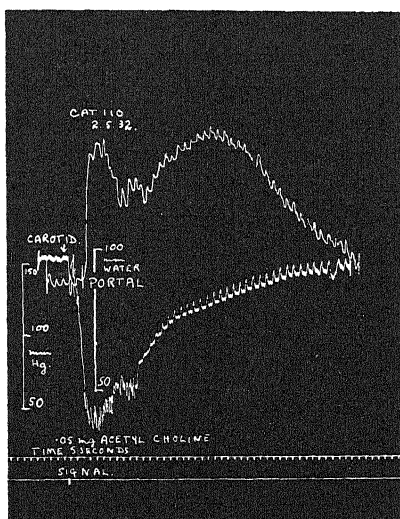


Fig. 18. Upper record: carotid pressure. Lower record: portal pressure. (Tracings subsequently cross.) Time record: 5 sec. Plethora had been induced by means of the injection of gum saline. Acetylcholine then brings about a rise of portal pressure in place of the usual fall.

have not found any evidence of change in calibre (r) of the portal venules within the liver, and the viscosity of the blood (η) presumably remains unaltered, so that

$$V = pK, \text{ or } V \propto p.$$

A diminution of the volume flow from 136 to 99 would therefore account for a drop in portal pressure from 80 to 58 mm. water. This approximates nearly to the fall of 20 mm. water which was observed in the experiments.

With the compensator communicating with the circulation (Figs. 12,

13), the initial rise in portal pressure may amount to 70–100 mm. water, and yet the only increase of inflow that could be detected was from 64 to 75 drops per minute (Fig. 14). This would account for a rise of portal pressure of only 15 mm. water. It would appear probable that the rise in general venous pressure produced by the use of the compensator with acetylcholine (Fig. 17) is responsible for the remainder of the rise in portal pressure.

From the same argument, part of the increase in liver volume brought about by the compensator is probably due to the rise of pressure in the vena cava.

As shown in Fig. 16, when the vascular system has been overfilled following the use of the compensator, acetylcholine may continue to cause an initial rise in vena cava pressure: in the same way a rise in portal pressure may also be brought about (Fig. 18). This rise takes the place of the usual fall in portal pressure and is followed by a secondary rise.

It is thus seen that acetylcholine in the presence of plethora of the circulation, may bring about a rise in general venous pressure in which the portal system shares. The type of curve then produced (Fig. 17) is mentioned particularly, as it was occasionally seen in cats (2 out of 70) where there had been no artificial overfilling of the vascular system. In three experiments carried out in dogs, this type of portal pressure response to acetylcholine was present in all.

(h) The secondary rise in portal pressure.

In all animals where the circulatory conditions were good and the blood-pressure well maintained, a well-marked secondary rise in portal pressure was seen following the injection of acetylcholine. This rise begins when the arterial pressure is beginning to recover and, generally speaking, the deeper the trough of arterial pressure and the steeper the subsequent rise, the more pronounced the secondary rise in portal pressure (Fig. 4).

The fact, seen from the records of outflow from the mesenteric vein (Fig. 7), that the portal pressure is rising when the inflow is markedly diminished, renders it obvious that the secondary rise in portal pressure must take place by a diminution of outflow from the portal system. It has also been noted that the cardiac slowing, brought about by acetylcholine, is often followed by a period of increased pulse-rate, above the original level. These observations led to the thought that the sympathetic might play a part in the recovery of blood-pressure following its depression by acetylcholine. This hypothesis was put to the test by the use of ergotamine

and ergotoxine to paralyse the sympathetic. Fig. 19 shows the result of this procedure.

It is seen that when the sympathetic is paralysed, the heart beats more slowly as a result of some loss of sympathetic tone. The injection of adrenaline into the portal system no longer leads to the immediate rise in portal pressure which would result with an intact sympathetic system. No significant change is seen in the portal pressure (? no sympathetic

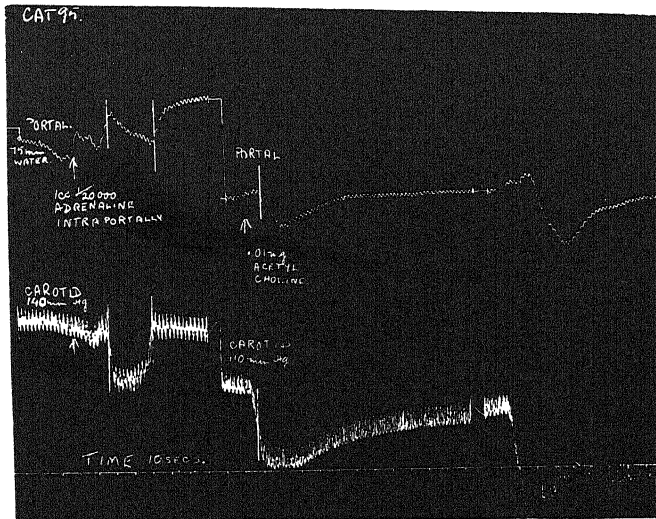


Fig. 19. Upper record: portal pressure. Lower record: carotid pressure. Time record: 10 sec. Following the administration of 20 mg. ergotoxine, 1 c.c. 1/20,000 adrenaline intraportally causes little significant change in the portal pressure, and, a few moments later, a falling carotid pressure results. Acetylcholine then brings about a fall in carotid pressure with gradual recovery and a fall in portal pressure with no secondary rise.

dilator fibres), and the action of adrenaline on the arterial system is to bring about a fall in pressure. The injection of acetylcholine into the animal then brings about the usual fall in arterial pressure, accompanied by a fall in portal pressure analogous to the initial fall in the intact animal. The recovery from the depression of the arterial pressure is slow and gradual, while the secondary rise in portal pressure is absent. The conditions in this animal, and in that illustrated in Fig. 20, are such that a secondary rise of portal pressure might have been anticipated.

In the previous paper [McMichael, 1932], it was pointed out that the splanchnic vessels in the failing preparation were in a condition of greatly

increased tone, which accounted for the absence of any further contraction with adrenaline. In particular, the adrenaline rise in portal pressure normally resulting from the constriction of the portal ramifications within the liver was absent. It is probable that the absence of the secondary rise of portal pressure in the animal with a failing circulation is due to the same circumstance. (See Fig. 11.)

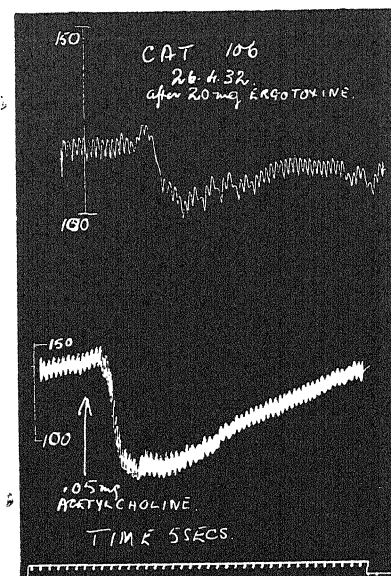


Fig. 20. Upper record: portal pressure. Lower record: carotid pressure. Time record: 5 sec. After 20 mg. ergotoxine the reaction to adrenaline had been reversed. 0.05 mg. acetylcholine brought about a fall in arterial pressure followed by a fall in portal pressure. The recovery of the arterial pressure takes place at a uniform slow rate and there is no secondary rise in portal pressure.

The evidence therefore suggests that the secondary rise in portal pressure after acetylcholine is dependent on the integrity of the sympathetic system. When this is thrown out of action by ergot the rise does not take place. The secondary rise does not occur when the sympathetic tone has been increased as a result of a failing circulation.

It is also to be noted that in the animal with paralysis of the sympathetic system, the rate of recovery of the arterial pressure from the acetylcholine depression is slower than in the animal with normal circulatory reactions, such as that in Figs. 4 and 5. The recovery rate is also

slow in the presence of a failing circulation. The association of the period of rapid recovery (Fig. 7), with a diminution of flow through the splanchnic arterioles, is evidence of splanchnic vaso-constriction at this stage. All these points are in favour of the hypothesis that the sympathetic plays a part in the recovery of the blood-pressure after acetylcholine. Depending on the degree of sympathetic stimulation elicited, so does the secondary rise in portal pressure vary in extent.

Thus acetylcholine resembles adrenaline in calling out the opposing autonomic mechanism at the height of its action. Reflex vaso-dilatation and cardiac slowing are elicited at the point of maximum adrenaline effect. Acetylcholine at its point of maximum activity calls forth cardiac acceleration and splanchnic vaso-constriction.

DISCUSSION.

The results obtained in this investigation add some confirmatory evidence to the view of Griffith and Emery [1930] that the vagus nerve does not carry vaso-motor fibres to the liver. These observers stimulated the vagus nerve, avoiding cardiac inhibition, and found no change in liver volume. The experiment described in Fig. 19 would indicate too, that as far as the portal vein is concerned there are no sympathetic dilator fibres. The vaso-dilator reflexes in the liver obtained by Griffith and Emery, were shown to be mediated through the splanchnics, and it is probable that any dilator reaction which occurs in the portal venules must be due to inhibition of vaso-constrictor tone.

Effects similar to those resulting from the intravenous injection of acetylcholine were obtained by Carnot, Gayet and Merklen (1930), by stimulation of the vagus. They found an initial fall in portal pressure, which they ascribed to diminished inflow into the portal system, and a rise of portal pressure, which occurred as the arterial pressure recovered.

So far as the cat is concerned, there are anatomical reasons brought forward by Popper [1931], which indicate that the "Lebersperre" mechanism described in the dog by Mautner and Pick, and by Bauer, Dale, Poulsson and Richards [1932] is absent. In the dog the muscular coat of the hepatic vein is much better developed than that in the cat, or the human subject. Dale and his colleagues did not find any clear evidence of vagus control of the liver circulation nor did they record any significant results following the injection of acetylcholine. Grab, Janssen and Rein [1929-30], showed an increased rate of flow through the liver with atropine. Inflow and outflow were simultaneously increased, and

thus there is no reason to assume any immediate vagus effect upon the liver circulation. Such effects as are observed are entirely secondary to alterations in the general circulation.

In the experiments detailed in this and in the preceding paper [1932], outflow from the liver has not been measured directly. All the vaso-motor reactions which we have obtained have been satisfactorily accounted for by the demonstrable changes in the circulation through the splanchnic vessels and the portal vein. In none of the experiments has there been any evidence of a controlling mechanism at the outlet of the liver lobule. This is in complete agreement with the observations made by Dale and his collaborators on the liver of the cat.

SUMMARY.

In the cat:

1. Large doses of acetylcholine bring about a rise in portal pressure by constricting the portal vein.

2. (a) With smaller doses of acetylcholine there is no direct constricting action on the portal vein. Two phases are usually seen in the portal pressure curve, viz. an initial fall, and a secondary rise of pressure.

- (b) The initial fall in portal pressure is determined by diminished inflow into the portal system, due to the predominance of vaso-dilatation in the limbs, and to diminished cardiac output.

- (c) The diminished flow through the splanchnic arterioles, and the consequent fall in portal pressure, can be reversed by the Bayliss mercury compensator.

- (d) During the stage of cardiac slowing, the general venous pressure may rise. This rise is exaggerated in animals which have been rendered plethoric; in such animals the portal pressure may rise in parallel with the general venous pressure, and the initial portal pressure fall is not seen.

- (e) The secondary rise in portal pressure is due to constriction of the portal venules within the liver by the action of the sympathetic. It is absent when the sympathetic has been paralysed by ergot, and in the presence of a failing circulation.

3. There is no evidence of any parasympathetic dilator action on the portal or hepatic venules. The experiments with ergot also yielded no evidence of sympathetic dilator fibres to the portal vein.

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THE NERVOUS CONTROL OF THE CAUDAL REGION OF THE LARGE BOWEL IN THE CAT.

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To find the influence of a nervous outflow on an organ in the body one may either cut the nerve, with subsequent stimulation of the cut ends, or one may simply divide the nerve. In the former case the response of the organ is to nerve impulses set up by an abnormal stimulus which may elicit impulses in quantity and in quality different from the normal, while in the latter case, after the disturbances due to trauma have subsided, the response exhibited by the viscus is due to the absence of normal impulses.

The latter method has found little favour in the past. Barrington [1915], however, used just such a method, often in acute preparations, to elucidate the normal nervous control of the urinary bladder. The close developmental and physiological connection between the large bowel and the urinary bladder suggests that a similar method may be successful in investigating the normal nervous control of the large bowel.

NOMENCLATURE.

Because of present uncertainty about the constitution and function of the nervous outflows to the large bowel the purely morphological terms "lumbar" and "sacral" are used in preference to "sympathetic" and "parasympathetic" respectively. The names applied to the subdivisions of the outflows are those recommended by Langley and Anderson [1896]. The lumbar outflow arises from the second, third, fourth and sometimes fifth lumbar nerves. The fibres pass without interruption through the sympathetic chains and run as four or five strands on either side to the inferior mesenteric ganglia. These strands are the spinal rami of the inferior mesenteric ganglia. The inferior mesenteric ganglia, usually four in number, form a ring round the inferior mesenteric artery about $1\frac{1}{2}$ cm. from the origin of the artery from the aorta. The majority of the fibres of the lumbar outflow form synapses in these ganglia. From the ganglia arise the lumbar colonic nerves which accompany the inferior mesenteric artery to the colon. There also arise from the inferior mesenteric ganglia the hypogastric nerves which run caudad to join the pelvic plexus.

The sacral outflow consists of the pelvic nerves which usually arise from the second and third sacral nerves. The pelvic nerves divide after a short course into a cranial and caudal branch on each of which there is a conspicuous ganglion. From the caudal ganglion arise four or more branches which run dorsad to the colon as the sacral colonic nerves. The fibres of the sacral outflow have synapses close to the bowel itself.

Fig. 1, drawn from the description by Langley and Anderson [1896], illustrates diagrammatically the outflows to the large bowel.

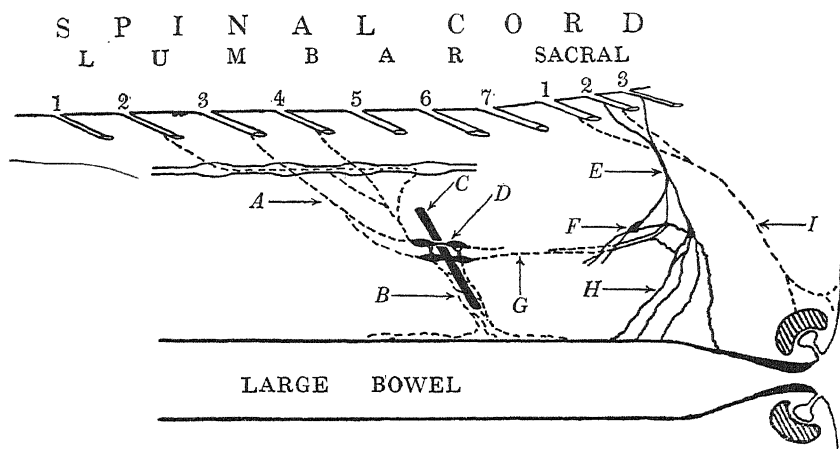


Fig. 1. Diagram of the outflows to the large bowel in the cat. *A*, spinal rami to the inferior mesenteric ganglia; *B*, lumbar colonic nerves; *C*, inferior mesenteric artery; *D*, inferior mesenteric ganglia; *E*, pelvic nerve; *F*, pelvic plexus; *G*, hypogastric nerve; *H*, sacral colonic nerves; *I*, pudendal nerve.

PREVIOUS WORK.

Bayliss and Starling [1900] and Elliott and Barclay-Smith [1904] found that the colon was always inactive after opening the peritoneum. Pithing the lumbo-sacral cord or dividing the nervous outflows to the large bowel led to activity of the gut. Lehmann [1913] noted increased colonic movement in dogs after division of the spinal rami to the inferior mesenteric ganglia. Markowitz and Campbell [1927] showed that spinal anaesthesia in dogs removes inhibition of the small intestine produced by intense peritoneal irritation, and in one of their five cases they thought that the movements of the colon were also augmented. Learmonth and Markowitz [1930] found that division of the lumbar colonic nerves in dogs increased colonic activity. This increase in activity was apparently greater when the pelvic nerves were intact. In these experiments, unfortunately, the peritoneum had been opened before section of the lumbar colonic nerves. Rankin and

Learmonth [1930] recommend division of the inferior mesenteric (lumbar colonic) nerves and of the presacral (hypogastric) nerves in man for the relief of megacolon.

Kuré [1931] and his fellow workers, however, cut the rami communicantes to the lumbar sympathetic chain on both sides in four dogs. The caudal part of the small intestine and the large intestine relaxed. Application of nicotine solution to the ganglia of the lumbar sympathetic chain on both sides in four dogs led to contraction of the large intestine and of the distal part of the small intestine. These results are supposed to be due to the presence of "spinal parasympathetic" fibres in the lumbar outflow. These fibres have no synapses, as have the true "sympathetic" fibres, in the lateral ganglia. In addition, stimulation of the lumbar sympathetic chain gave motor responses from the colon both in cats and in dogs with or without the use of nicotine.

Division of both pelvic nerves by Elliott [1906] apparently did not lead to gross atony of the colon in cats because faeces did not accumulate in the large bowel. Barrington [1915] observed dilatation of the large bowel in cats after cutting the dorsal roots of the sacral nerves. Adamson and Aird [1932] found that section of the pelvic nerves in cats led, after an interval of several weeks, to megacolon.

METHODS.

The cats were decerebrated under ether anaesthesia and no observations were carried out for at least 1 hour after decerebration. In a few cases decapitation or anaesthesia with one of the barbituric derivatives was substituted for decerebration. Faecal matter was removed from the large bowel after decerebration by means of a soap and water enema. The animals received a meal of porridge and milk on the morning of the day of the experiment some 2 hours before anaesthetization.

The behaviour of the large bowel was recorded by a thin rubber balloon of large diameter inserted through the anus. The balloon holder, 22 cm. long, was a thin copper tube with numerous perforations in the terminal 4 cm. where the balloon was attached. The copper tube was marked off in cm. so that the position of the balloon within the gut could be gauged. Usually the caudal end of the balloon lay from 4 to 6 cm. cranial to the anal canal. The balloon holder was firmly grasped by a clamp attached to the operating table.

Volume recording at a constant pressure of 21 cm. water was used. The tonicity of the gut was estimated by recording the volume of fluid

accepted by the balloon in the gut at constant pressure. The rhythmical behaviour of the gut appeared on the record after release of the pressure. Several consistent records were obtained before interfering with the nervous outflows to the gut.

The lumbar outflow was approached extraperitoneally through a left flank incision. The hypogastric nerves were cut without opening the peritoneum, but the peritoneum had to be opened in order to divide the spinal rami to the inferior mesenteric ganglia and also to cut the lumbar colonic nerves. The pelvic nerves were exposed and cut extraperitoneally by incisions along the inguinal canals.

The lumbo-sacral cord was isolated by tying the spinal cord in the lower thoracic region. Full ether anaesthesia was used and ample time allowed for disappearance of the ether before continuing the observations. Such cord section is craniad to the roots of origin of the lumbar and sacral outflows in cats.

Spinal anaesthesia was induced by intrathecal injection of 1 to 2 c.c. of a 1 p.c. "Novocain" solution craniad or caudad to the last lumbar spinous process. As tested by the knee jerk, anaesthetization of the cord lasted for from 30 to 60 minutes.

RESULTS.

(1) *The effect of division of the lumbar outflow.* When the lumbar outflow is intact the large bowel is inactive. Section of the entire lumbar outflow leads to increase in tone and in rhythmical activity (Fig. 3, C, 1, 2). Section of the spinal rami to the inferior mesenteric ganglia alone causes a slight increase in tone and the appearance of rhythmical contraction. Subsequent section of the lumbar colonic nerves and of the hypogastric nerves leads to marked increase both in tone and in rhythmicity (Fig. 2, B). Division of the hypogastric nerves alone has little effect, but section of the lumbar colonic nerves, even when the hypogastric nerves are intact, leads to this marked increase in gut activity (Fig. 2, A, C, 1, 2, 3). Such results occur after isolation of the lumbo-sacral cord and the integrity of the pelvic nerves seems to make little difference to the result.

(2) *The effect of division of the sacral outflow.* In five cats the sacral nerve roots or the pelvic nerves themselves were cut and the animals allowed to recover. These animals experienced difficulty in emptying the large bowel and post-mortem the colon was dilated.

In acute preparations, when the lumbar outflow is intact, division of the pelvic nerves may lead to further relaxation of the large bowel

(Fig. 3, A, 2, 3). Such relaxation is rarely marked but, when the lumbar outflow has previously been cut, and the gut in consequence in a state

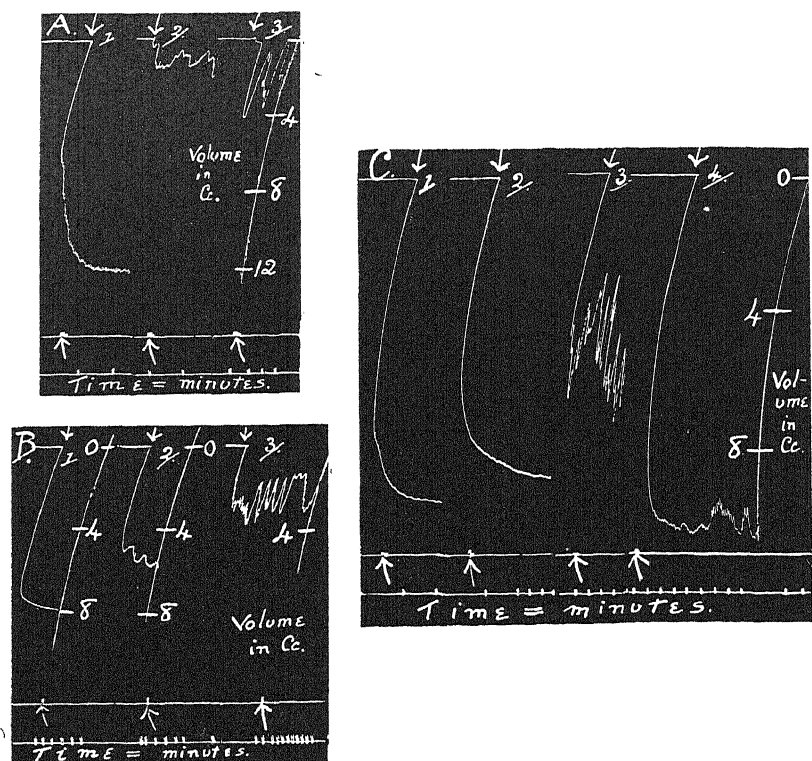


Fig. 2.

Series A. Cat: decerebrate; pudendal nerves cut. 1. Pressure released at 21 cm. H₂O. 2. Lumbar colonic nerves cut; pressure released as before. 3. Hypogastric nerves cut; pressure released as before.

Series B. Cat: decerebrate; pudendal nerves cut; lumbo-sacral cord isolated; pelvic nerves cut. 1. Pressure released at 21 cm. H₂O. 2. Spinal rami to inferior mesenteric ganglia cut; pressure released as before. 3. Lumbar colonic and hypogastric nerves cut; pressure released as before.

Series C. Cat: decerebrate; pudendal nerves cut. 1. Pressure released at 21 cm. H₂O. 2. Hypogastric nerves cut; pressure released as before. 3. Lumbar colonic nerves cut; pressure released as before. 4. Pelvic nerves cut; pressure released as before.

of high tone and activity, section of the pelvic nerves then produces an unequivocal fall in tone and possibly some decrease in the rate of rhythmical contraction (Fig. 2, C, 3, 4). After the division of the pelvic nerves the decrease in resistance to passage of the recording balloon craniad

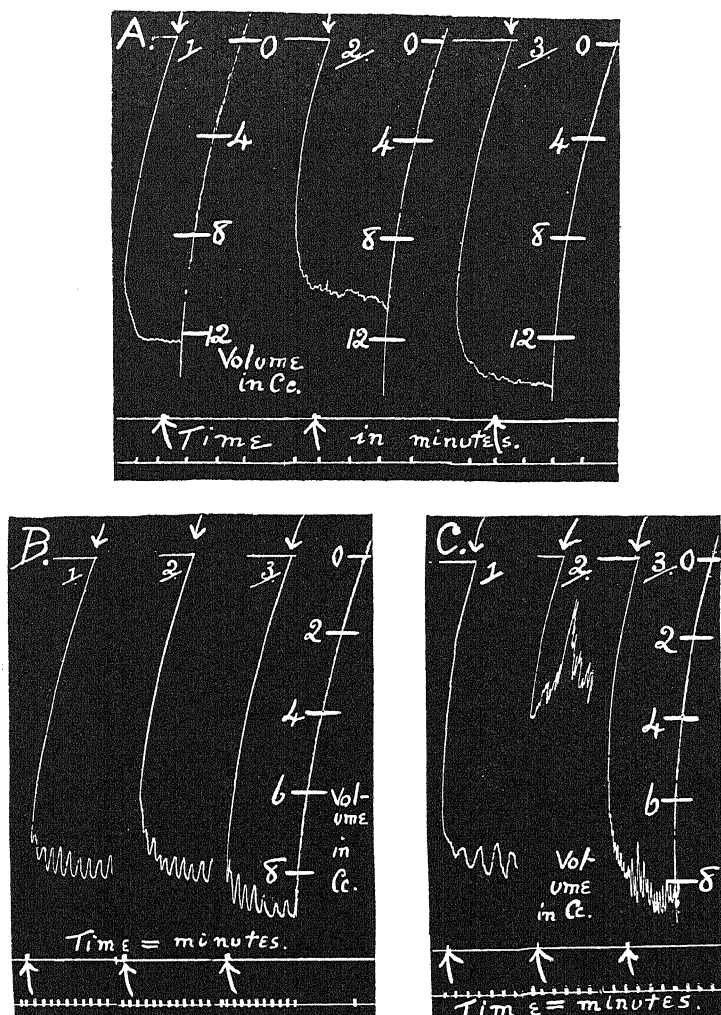


Fig. 3.

- Series A. Cat: decerebrate. 1. Pressure released at 21 cm. H₂O. 2. Pudendal nerves cut; pressure released as before. 3. Pelvic nerves cut; pressure released as before.
- Series B. Cat: decerebrate; lumbar outflow cut; lumbo-sacral cord isolated. 1. Pressure released at 21 cm. H₂O. 2. Pelvic nerves exposed; pressure released as before. 3. Pelvic nerves cut 3 hours after cord transection; pressure released as before.
- Series C. Cat: decerebrate; pudendal nerves cut. 1. Pressure released at 21 cm. H₂O. 2. Lumbar outflow cut; pressure released as before. 3. Cord tied in lower thoracic region; pressure released as before.

along the gut may be very obvious. The fall in tone of the gut is certainly not due to the operative interference necessary to expose the pelvic nerves.

Shortly after isolation of the lumbo-sacral cord, division of the pelvic nerves, although the lumbar outflow be cut, is usually without effect. Cutting the pelvic nerves several hours after transection of the cord may, however, give some indication of relaxation of the large bowel (Fig. 3, B).

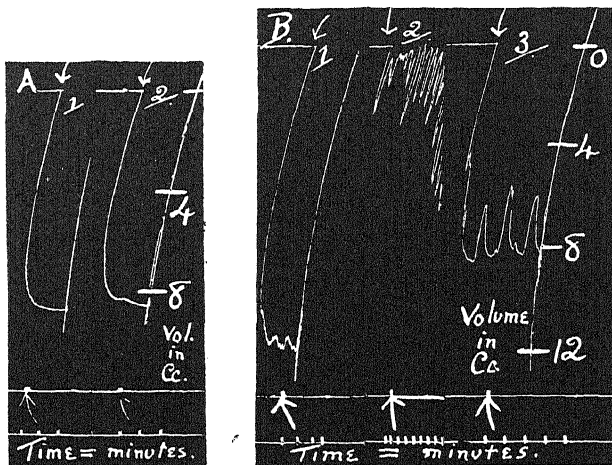


Fig. 4.

Series A. Cat: Na amytal anaesthesia-55 mg./kg.-pudendal nerves cut; pelvic nerves cut.

1. Pressure released at 21 cm. H_2O . 2. Cord tied in lower thoracic region; pressure released as before.

Series B. Cat: Na amytal anaesthesia-56 mg./kg. 1. Pressure released at 21 cm. H_2O .

2. 1 c.c. 1 p.c. "Novocain" injected intrathecally; pressure released as before 10 min. after injection. 3. Pressure released as before 60 min. after injection.

(3) *The effect of isolation of the lumbo-sacral cord.* When the lumbar outflow is intact and the pelvic nerves divided, isolation of the lumbo-sacral cord causes no significant alteration in the large bowel; there is certainly no contraction (Fig. 4, A). When, however, the lumbar outflow is cut and the sacral outflow intact, transection of the spinal cord leads to obvious relaxation of the large bowel (Fig. 3, C, 2, 3). Even when both outflows are intact, isolation of the lumbo-sacral cord may cause slight relaxation of the large bowel.

(4) *The effect of spinal anaesthesia.* When both outflows to the large bowel are intact, spinal anaesthesia invariably leads to increased activity

of the gut. The result is similar after division of the sacral outflow and after isolation of the lumbo-sacral cord.

If the lumbar outflow be cut and the sacral outflow left intact, spinal anaesthesia leads to relaxation of the gut, very apparent before section of the cord but slight after isolation of the lumbo-sacral cord. The possibility that spinal anaesthesia indirectly affects the volume of fluid accepted by the gut by causing flaccidity of the abdominal and pelvo-caudal muscles throws some doubt on such observations, although, after division of both outflows to the large bowel, spinal anaesthesia does not have any obvious effect on the record from the gut.

The effect of spinal anaesthesia on the large bowel of an anaesthetized cat with both outflows intact is shown in Fig. 4, B.

When both outflows to the gut are intact, or when only the lumbar outflow is intact, division of the pudendal nerves, in practically every case, leads to slight but definite increase in the activity of the large bowel (Fig. 3, A, 1, 2).

DISCUSSION AND CONCLUSIONS.

The lumbar outflow exerts a constant inhibitory influence on the large bowel, and this inhibition is apparently more powerful than the motor tone passing by the sacral outflow. If the peritoneal cavity be opened beforehand, such powerful inhibition is understandable, and division of any part of the lumbar outflow can give no true indication of the normal impulses passing along the lumbar outflow. In the present series, however, the integrity of the peritoneal cavity was jealously guarded before division of the lumbar outflow. It may be that the marked inhibition is due to the recent operative interference in acute preparations. On the other hand, the motor influence through the sacral outflow may be intermittent, as the work of Hertz and Newton [1913] on the gastro-colic reflex suggests. Such intermittent activity, however powerful, simple nerve section cannot be expected to detect.

A constant motor influence of the sacral outflow, however, may be detected by previous division of the lumbar outflow, but this does not seem to be powerful in acute experiments, and, even in survival experiments, division of the sacral outflow apparently does not lead to rapid or gross atony of the large bowel. It is possible that the dilatation of the large bowel observed by Barrington [1915] and by Adamson and Aird [1932], after interference with the sacral outflow, is due to distension of the gut as the result of the absence of the normal expulsive responses which depend so largely on the integrity of the pelvic nerves [Garry, 1932].

The central inhibitory tone for the large bowel seems to be confined to the lumbo-sacral cord. In this the colon differs from the urinary bladder where part of the inhibition in the lumbar outflow arises craniad to the lumbar cord [Barrington, 1915]. It is difficult to decide if the inhibitory influence of the inferior mesenteric ganglia is a normal phenomenon or not. Barrington [1915] also found that the inferior mesenteric ganglia act as an extraspinal centre of inhibition for the bladder. Division of the spinal rami to the inferior mesenteric ganglia alone certainly never causes such marked increase in colonic activity as division of the lumbar colonic nerves. This may be due to failure to cut all the spinal rami to the inferior mesenteric ganglia, but it is possible that the inhibitory influence exerted by such an extraspinal centre is the result of the local interference at the time of section of the spinal rami. The marked increase in gut activity following spinal anaesthesia, in effect division of the spinal rami without local interference, suggests that the inhibitory powers of the inferior mesenteric ganglia are acquired in response to an abnormal situation. Distal to the inferior mesenteric ganglia the inhibitory impulses follow the lumbar colonic nerves almost exclusively. The persistence of the inhibition after division of the hypogastric nerves is not due to switching of impulses, normally passing down the hypogastric nerves, to the lumbar colonic nerves, because initial division of the lumbar colonic nerves leads to an increase in bowel activity little, if at all, augmented by subsequent section of the hypogastric nerves. Stimulation of the peripheral ends of the cut hypogastric nerves causes obvious inhibition of the colon which shows the danger of drawing conclusions from the results of nerve stimulation alone.

The motor influence through the sacral outflow arises mainly craniad to the lumbo-sacral cord. There is some slight evidence, even in acute preparations, for a secondary motor centre in the isolated lumbo-sacral cord. Such conclusions are similar to those of Barrington [1915] for the urinary bladder.

The slight increase in activity of the large bowel after division of the pudendal nerves may be due to removal of afferent impulses in the pudendal nerves set up by irritation of the anal canal. Such impulses, like other afferent impulses, may tend to cause inhibition of the gut.

SUMMARY.

The behaviour of the caudal end of the large bowel in cats is recorded by means of a balloon introduced through the anus.

In acute decerebrate cats the gut is invariably inactive. This inactivity is due to inhibitory impulses, arising locally in the lumbo-sacral cord, passing out to the large bowel by way of the spinal rami to the inferior mesenteric ganglia and then by the lumbar colonic nerves. The hypogastric nerves do not normally transmit such impulses to the gut. After section of the spinal rami the inferior mesenteric ganglia still exert an inhibitory influence on the large bowel. The motor influence exerted by the sacral outflow is not strong and arises mainly cranial to the lumbo-sacral cord. There is some evidence for a subsidiary motor centre in the isolated lumbo-sacral cord.

Division of the pudendal nerves causes a slight increase in the activity of the large bowel.

I am deeply indebted to Prof. E. P. Cathcart for constant interest and encouragement in this work.

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THE INFLUENCE OF OXYGEN PRESSURE ON
THE METABOLISM OF THE ISOLATED
COLD-BLOODED HEART.

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THE authors found that the respiratory quotients of isolated hearts of frogs and of tortoises were lower when the hearts were suspended in pure oxygen than when suspended in air. Furthermore the R.Q. in oxygen in most cases was less than 0.8, a fact which suggested the possibility of the hearts utilizing fat. The experiments described below were made in order to analyse the metabolic exchange of hearts working in air and in oxygen, in the hope of explaining these phenomena.

METHODS.

Rana esc. (Hung.) and *Testudo graeca* were used. The oxygen consumption and carbon dioxide production were measured by the method already described by the authors [1931]. The Barcroft apparatuses used had large bulbs with a capacity of about 400 c.c. and two or three frogs' hearts or one tortoise heart were used in each experiment. The frogs' hearts were perfused by the method previously described [1931] and the tortoise hearts were perfused by a similar method, with two cannulae (venous and aortic) which were arranged to provide a circulation. In some experiments the tortoise auricle alone was used, and was prepared by the method described by Clark and White [1930]. The Ringer's fluid used was buffered with phosphate, and was of the composition previously described [1931].

The general method adopted was to allow 2 hours for equilibrium to be established after the hearts had been set up in the Barcroft apparatus, and to take readings for the subsequent 4 hours. In order to estimate the total oxygen usage, the oxygen consumption during the first 2 hours was

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calculated by extrapolation from the later figures. It was found that irregular readings were obtained for both oxygen consumption and CO_2 production during the first hour, but that between the first and second hours both these figures attained steady values; these were maintained until the end of the experiment provided that the hearts remained in good condition. Experience showed that reliable measurements of the R.Q. could be obtained if the hearts maintained a fairly uniform activity for 6 hours, but that with the large bulbs used in these experiments readings over shorter periods gave unreliable results. The values obtained for the R.Q. also were found to be unreliable when there was any progressive impairment of the hearts' activity, such as was produced by oxygen lack or by poisoning with iodo-acetic acid.

The chemical methods used have been described in previous papers by the authors: estimation of total carbohydrate [1931]; estimation of lactic acid [1932 b]; a new method was used for the estimation of fat, namely that described by Stewart, Gaddie and Dunlop [1931]. This is a titrometric method that estimates the number of carboxyl groups in precipitable fatty acids.

THE EFFECT OF OXYGEN PRESSURE ON THE RESPIRATORY EXCHANGE.

The first object of the experiments was to determine the effect on the R.Q. of substituting pure oxygen for air. In a series of experiments all conditions were kept exactly constant, except that on alternate days the Barcroft bulbs were filled with pure oxygen instead of air. Any differences observed cannot therefore be due to any constant experimental error, nor to any change in the condition of the frogs.

Table I shows the fundamental result dealt with in this paper. In the four sets of experiments shown in the table, the R.Q. was in every case lower in oxygen than in air, and the difference was considerable (from 0.17 to 0.12).

The figures obtained in air may be compared with the following figures previously obtained by the authors, with frogs' hearts perfused for 6 hours and suspended in air. (The values for oxygen consumption have been converted to c.c. per g. per hour.) Perfused Ringer's fluid: (1931) 21 hearts, 1.26 c.c. O_2 (R.Q. 0.86); (1932 a) 11 hearts, 1.39 c.c. O_2 (R.Q. 0.89). Perfused Ringer's fluid with 25 p.c. serum: (1931) 8 hearts, 1.9 c.c. O_2 (R.Q. 0.84). Perfused with heparinized frog's blood: (1931) 7 hearts, 1.51 c.c. (R.Q. 0.90).

The present results show a lower oxygen consumption in Ringer's fluid, and a slightly higher R.Q. than do our previous figures. The only other difference worth noting is that in our previous work we found a slightly lower R.Q. with Ringer serum than with Ringer's fluid, whereas the present results show the former figure slightly greater than the latter. The two sets of results indicate that there is no significant difference in the R.Q. in Ringer's fluid with and without the addition of plasma or serum.

TABLE I. Respiratory exchange in air and in oxygen.
(Duration of experiments 6 hours.)

System	Air				Oxygen			
	No. of hearts	R.Q.	Oxygen used (c.c. per g. per hr.)	CO ₂ produced (c.c. per g. per hr.)	No. of hearts	R.Q.	Oxygen used (c.c. per g. per hr.)	CO ₂ produced (c.c. per g. per hr.)
Frogs' hearts.								
1. Ringer's fluid	34	0.903	0.80	0.72	39	0.730	0.90	0.66
2. Ringer's fluid + 10 p.c. frog's plasma (heparinized)	12	0.913	1.18	1.08	24	0.760	1.35	1.03
3. Ringer's fluid + 50 p.c. frog's blood (heparinized)	8	0.902	1.92	1.73	12	0.793	2.28	1.81
Tortoises' hearts.								
Ringer's fluid + 10 p.c. tortoises' serum	5	1.035	0.46	0.50	4	0.915	0.69	0.63

The figures for frogs' hearts in Table I show the effect of substitution of pure oxygen for air in hearts perfused with Ringer's fluid, with Ringer plasma, and with Ringer blood. This change caused an increase of oxygen consumption in all cases, whilst the CO₂ production decreased about 5 p.c. in two cases and increased about 5 p.c. in the third case. In the case of the tortoise heart both the oxygen consumption and the CO₂ production were increased considerably by substitution of pure oxygen for air.

These results indicate that oxygen causes the hearts to metabolise some substance with a low R.Q. The chief probable source of error affecting this conclusion is a variable production of lactic acid, for the acid might liberate preformed CO₂ from the system, and if more acid were produced in air than in oxygen this might account for some or all of the differences observed in the R.Q.

LACTIC ACID PRODUCTION.

The perfusion fluid when introduced contained no carbonate but Clark and White [1928*b*] found that during perfusion an exchange of ions took place between the heart and the perfusion fluid; either phosphate or chloride ions passed into the heart and were replaced by carbonate ions. The present authors found [1931, Table XIII] that frogs' hearts after perfusion with phosphate Ringer's fluid for 6 hours lost about

0.15 c.c. CO₂ per g., and that the perfusion fluid took up about 0.25 c.c. CO₂ per g. of heart. In our present experiments readings were commenced after 2 hours' perfusion, and at this time sufficient CO₂ would be present in the fluid for a measurable amount to be liberated by any lactic acid produced.

TABLE II. Lactic acid produced in 6 hours by perfused hearts.

System	No. of hearts	Air			No. of hearts	Oxygen		
		Lactic acid produced (mg. per g.)	CO ₂ equivalent of lactic acid produced (c.c. per g.)	Total CO ₂ produced (c.c. per g.)		Lactic acid produced (mg. per g.)	CO ₂ equivalent of lactic acid produced (c.c. per g.)	Total CO ₂ produced (c.c. per g.)
Frogs' hearts								
1. Ringer's fluid	20	0.564	0.14	4.32	22	0.296	0.074	3.96
2. Ringer's fluid + 10 p.c. frogs' plasma	8	0.550	0.14	6.48	8	0.530	0.130	6.18
Tortoisés' hearts								
Ringer's fluid + 10 p.c. tortoisés' serum	10	2.180	0.54	3.00	8	0.156	0.039	3.78

Table II shows the amount of lactic acid excreted by hearts under various conditions. The authors have shown [1932 *a*] that the lactic acid content inside the heart does not rise during perfusion. The maximum amounts of CO₂ that could be liberated by the lactic acid excreted are shown in Table II. These quantities are calculated from the formula

$$1 \text{ mg. lactic acid} = \frac{22.4}{90} = 0.25 \text{ c.c. CO}_2.$$

With regard to the figures obtained it may be noted that the value of 0.564 mg. lactic acid produced per g. heart in 6 hours obtained with hearts perfused with Ringer's fluid in air, is higher than the previous figure of 0.6 mg. per g. in 20 hours obtained by us (1932 *b*) with hearts perfused in the same manner. The significance of the figures for lactic acid production in hearts perfused with plasma is somewhat uncertain, for some production occurred in control fluids kept standing at room temperature for 6 hours.

Table II shows that in the case of frogs' hearts the lactic acid production in either air or oxygen did not exceed 0.56 mg. per g. and that the amount of CO₂ that this quantity could liberate was not more than about 3 p.c. of the total CO₂ production of the heart. Moreover the lactic acid production in air and oxygen was equal in the case of plasma perfusion, and in the case of Ringer perfusion the difference was only 0.27 mg. per g. In the case of the frogs' hearts therefore lactic acid

production could not produce any measurable error in the R.Q. estimations.

In the case of the tortoises' hearts there is a large difference in the lactic acid production in air and in oxygen. Subtraction of the CO_2 that might be liberated by the lactic acid would reduce the R.Q. observed in air from 1.035 to 0.892, a figure which is nearly the same as the R.Q. obtained in oxygen which is 0.903 when similarly corrected. Lactic acid production therefore provides an adequate explanation for the fact that an R.Q. above unity was obtained with the tortoise heart in air, and might account for the difference observed in the R.Q.'s obtained in air and in oxygen. The experiments on tortoises' hearts do not therefore provide conclusive evidence of a difference in the R.Q. in air and in oxygen, but in the case of frogs' hearts, the difference observed in these two conditions is not affected significantly by the lactic acid production.

CARBOHYDRATE METABOLISM IN AIR AND IN OXYGEN.

Estimations of the carbohydrate consumption of isolated hearts must be based on a comparison of the amount of total reducing substances present in hearts after perfusion with the amount present in unperfused controls. Unfortunately it is very difficult to obtain reliable control values, for the authors have found that the values obtained in the controls vary greatly not only as regards individual frogs, but equally as regards the averages of different batches, and also in the same batches of frogs after different periods of captivity. For example after a few weeks' captivity the amount of reducing substance in control hearts rises rapidly. Hence it not only is necessary to use large numbers of frogs as controls, but also it is necessary to make control estimations at the same time as the experiments are carried out. We have taken as full precautions as possible but feel that calculations based on comparisons with controls are somewhat uncertain.

The estimations of the content of total reducing substances in controls, shown in Table III were obtained from the following numbers: batch I, 19 hearts; batch II, 6 hearts; batch IV, 12 hearts. The standard deviation of the mean (σ_m) varied between 0.6 and 0.8. In the case of the averages for the perfused hearts, the σ_m lay between 0.5 and 1.

The figures for lactic acid excretion are taken from Table II. The figures for sugar excretion agree with the values we have obtained previously [1932 *a*, Table III]. As regards the calculations of gain and loss of reducing substances, the estimations of the standard variation of the averages indicates that differences of less than 2 mg. per g. are of doubtful significance.

The results shown in Table III provide fairly certain evidence that the hearts use more carbohydrate when working in air than when working

in oxygen. In the first place the R.Q. is higher in air than in oxygen, and in the second place all four pairs of averages given in Table III show a lower value for the total reducing substances in the heart after perfusion in air than after perfusion in oxygen. In all four cases the experiments compared were conducted on alternate days, and therefore the differences observed are believed to be significant.

TABLE III. Carbohydrate metabolism of frogs' hearts.

Batch no. of frogs	No. of hearts	Duration of exp. in hr.	Total O ₂ used (c.c. per g. heart)	R.Q.	Total reducing substance in hearts (mg. per g.)		Amounts excreted into (+) or removed from (-) perfusion fluid (mg. per g. heart)		Loss or gain of carbo- hydrate in the heart- fluid system (mg. per g. heart)
					(a)	(b)	(a)	(b)	
Perfused with Ringer's fluid in air.									
I <i>a</i>	9	6.0	4.50	0.93	15.22	12.48			-1.10
II <i>a</i>	9	6.0	5.90	0.87	8.90	5.71	+0.56	+1.08	-1.55
III <i>a</i>	16	6.3	4.46	0.90	—	7.75			—
Perfused with Ringer's fluid in oxygen.									
I <i>b</i>	12	6.0	4.33	0.74	15.22	15.49			+2.09
II <i>b</i>	15	6.0	6.15	0.75	8.90	7.31	+0.30	+1.34	+0.05
III <i>b</i>	12	7.3	6.66	0.70	—	7.97			—
Perfused with Ringer plasma in air.									
IV <i>a</i>	12	6.0	7.10	0.91	14.99	7.78	+0.55	-0.04	-6.60
Perfused with Ringer plasma in oxygen.									
IV <i>b</i>	12	6.0	7.16	0.74	14.99	10.41	+0.52	-0.10	-4.16
Perfused with Ringer's fluid in 95 p.c. N ₂ and 5 p.c. O ₂ .									
II <i>c</i>	12	6.0	2.94	—	8.90	5.66	+1.0	+1.84	-0.40
Perfused in oxygen with Ringer's fluid +0.001 p.c. iodo-acetic acid.									
II <i>d</i>	27	6.0	2.75	—	15.22	12.86	0	+1.78	-0.58

The estimations of total reducing substances indicate that hearts perfused with Ringer's fluid used very little carbohydrate when perfused in air, and the figures for the hearts perfused in oxygen actually show a gain in reducing substances. The significance of this apparent gain is uncertain but the problem has been discussed previously by the authors [1932 a].

A few experiments were made with reduced oxygen pressure (5 p.c. O₂ in N₂). These were inconclusive because heart block developed after a few hours. The writers have shown [1931] that a ventricle driven by electrical stimuli can maintain its activity unimpaired for hours under anaerobic conditions. This, however, is not true as regards the conductive mechanism of the heart which is very easily paralysed by deficiency of oxygen. Similarly iodo-acetic acid in low concentrations produced heart block after 2-3 hours. Clark, Eggleton and Eggleton [1932] found that the frog's ventricle, driven by electrical stimulation, maintained a

normal activity in air for 2 hours in the presence of 0.008 p.c. iodo-acetic acid. This acid appears therefore to poison the conductive mechanism of the frog's heart long before it affects the contractility of the ventricle. The experiments with iodo-acetic acid show very little change in the reducing substances of the heart, whilst the experiments with reduced oxygen pressure show a considerable excretion of lactic acid. Unfortunately in both cases the occurrence of heart block made it impossible to get reliable figures for the R.Q.

CARBOHYDRATE METABOLISM OF TORTOISE HEART.

Experiments were made with tortoise hearts in the hope that the use of a larger mass of material would lead to increased accuracy of analysis. On the whole the tortoise heart has been found less suitable than the frog's heart for the purposes of these experiments. The heart provided with circulation does not obtain an adequate oxygen supply when working in air, but appears to do so when suspended in oxygen. The isolated auricle filled with Ringer serum mixture and suspended in oxygen probably does not obtain an adequate oxygen supply. Unfortunately the tortoises' hearts vary even more widely than do frogs' hearts as regards their content of reducing substances.

The content of reducing substances was estimated in the unperfused ventricles of 18 tortoises from a single batch and the average value obtained was 17.7 mg. per g., but the individual values varied from 8.2 to 30.8. The standard deviation was 6.7 and the σ_m was 1.6. There was however a correlation between the content of reducing substances in the auricle and in the ventricle. These two values were estimated in 9 hearts and the average figures were auricle 15 and ventricle 16.7 mg. reducing substance per g., whilst the average divergence between auricle and ventricle was only 2.7 mg. The control values for total reducing substances shown in Table IV were obtained as follows. In the 1930 experiments the auricles were perfused and the ventricles analysed as controls. The figure for 1931 is the average of 21 estimations. In the case of the 1932 experiments the number of tortoises available was not sufficient for control estimations and the value of 23.3 is the average of the 10 highest values in the previous batch, and merely indicates the highest probable figure.

The experiments made in 1932 on tortoises' hearts (Table IV) confirm the experiments on frogs' hearts in that the hearts suspended in air show a lower content of reducing substances than do those suspended in oxygen. About half of this difference can be accounted for by the lactic acid produced by hearts working in air. The figures indicate that the tortoise heart working in oxygen uses little carbohydrate but uses more when working in air. The auricle suspended in oxygen may metabolize as much as two-thirds of its carbohydrate but we believe that the

tortoise's auricle even when suspended in oxygen suffers from oxygen lack. The results do not indicate any important difference between the metabolism of the tortoise's heart and that of the frog's heart.

TABLE IV. Carbohydrate metabolism of tortoises' hearts.

Date	No. of hearts	Duration of exp. in hr.	Total O ₂ used (c.c. per g. heart)	R.Q.	Total reducing substances in hearts (mg. per g.)		Amounts excreted into (+) or removed from (-) perfusion fluid (mg. per g. heart)		Loss or gain of carbohydrate in heart fluid system (mg. per g. heart)	
					(a)	(b)	(a)	(b)		
					Con- trol	Per- fused	Lactic acid	Sugar		
Whole heart perfused Ringer serum in air.										
1932	5	6	2.74	1.035	(23.3)	15.1	+2.46	+0.03	5.7	
Whole heart perfused Ringer serum in oxygen.										
1932	4	6	4.16	0.915	(23.3)	20.4	+0.17	+0.01	2.7	
Whole heart perfused Ringer's fluid in oxygen.										
1931	7	24	11.3	—	16.3	12.7	—	—	3.4	
Auricle filled with Ringer serum in oxygen.										
1930	7	24	10.5	—	18.0	17.3	—	-2.0	>11	

NITROGENOUS METABOLISM.

Table V shows the amount of non-protein nitrogen excreted into perfusion fluids by the hearts. The values are very similar to those obtained previously by the authors, who found that the nitrogen excretion by frogs' hearts perfused with Ringer's fluid in air for 6 hours was 0.52 mg. per g. [1931], and 0.42 mg. per g. [1932 *a*]. The nitrogen excretion in plasma appears to be lower than that in Ringer's fluid, but the values with plasma are obtained by difference between the nitrogen content of control and perfusion fluid, and are therefore less accurate than those with Ringer's fluid.

The tortoise's heart, on account of its greater bulk, provides a more favourable opportunity for measuring nitrogen excretion, and it will be seen that this is only half as great per unit weight with the tortoises' hearts as with frogs' hearts. A control experiment showed that the tortoise heart (unperfused) contained 0.12 mg. per g. of urea N. The quantity of nitrogen found in the perfusion fluid was considerably greater than this, and must therefore have been formed during perfusion and cannot have been simply washed out of the heart.

We have previously estimated [1932 *a*] that about 0.07 mg. per g. of nitrogen is excreted for every c.c. of oxygen consumed. If it be

assumed that the excretion of 1 mg. N_2 involves an oxygen use of 6 c.c., then the nitrogen excretions shown in Table V account for proportions of the total metabolism which vary from 18 to 66 p.c., whilst our previous estimate corresponded to 42 p.c.

TABLE V. Nitrogen excreted by perfused hearts.

TABLE V. Nitrogen excreted by perfused hearts.							P.c. of total metabolism accounted for by N ₂ excretion
System	No. of hearts	Total wt. in g.	Urea N + NH ₃ -N (mg. per g. heart)			N excretion in mg. per c.c. of O ₂ used	
			(a) Control	(b) Perfusion fluid	(c) Difference		
Frogs' hearts.							
(a) Perfused Ringer's fluid							
(i) In air for 6 hr.	6	0.79	0	0.54	0.54	0.11	66
(ii) In oxygen for 6 hr.	12	1.33	0	0.59	0.59	0.11	66
(b) Perfused Ringer plasma mixture							
(i) In air for 6 hr.	4	0.47	0.75	1.02	0.27	0.04	24
(ii) In oxygen for 6 hr.	16	2.26	0.39	0.59	0.20	0.03	18
Tortoises' hearts.							
Perfused Ringer's fluid							
(i) In oxygen for 6 hr.	5	5.01	0	0.20	0.20	0.05	30
(ii) In oxygen for 24 hr.	5	5.01	0	0.42	0.42	0.04	24

FAT METABOLISM.

The fat content of the frog's heart was measured by the method of Stewart, Gaddie and Dunlop [1931] which estimated only the fatty acids, and this gave a value of about 1.5 p.c. fat. We previously [1931] estimated the fat content as 4.8 p.c., but the method employed [Stewart and White, 1925] included in the figure for fatty acids the phosphoric acid derived from phospholipins, and probably also some lactic acid formed from sugar during the alkaline hydrolysis.

TABLE VI. Fat metabolism of frogs' hearts perfused for 6 hours.

System	No. of hearts	Fat content of hearts		Fat removed from perfusion fluid (mg. per g. heart)
		Average as p.c.	σ_m of average	
Unperfused controls	20	1.34	0.07	—
Perfused in oxygen				
Ringer's fluid	8	1.35	0.19	—
Ringer plasma	12	1.64	0.08	1.56
Ringer blood	12	1.70	0.09	—

We previously concluded that analyses failed to show any loss of fat after 24 hours' perfusion. Our present experiments also show no loss after 6 hours' perfusion. Indeed the experiments with plasma and blood actually show a gain in fat, and since this is about four times the standard deviation of either of the means compared, it appears to be a significant difference. Analyses of the fluids showed a slight loss of fat from the plasma perfused through the heart with no appreciable alteration in the total fatty acid content of the system heart + perfusion fluid.

The analyses therefore suggest that the heart takes up fat when this is present in the perfusion fluid, but they do not provide any support for the hypothesis that the hearts oxidize fats.

Partial desaturation of the fatty acids of the heart would account for some oxygen consumption, without the liberation of any CO_2 and without any apparent reduction in the fat content of the hearts. Table VII shows,

TABLE VII. Effect of perfusion on iodine number of fats in frogs' hearts.

System	No. of hearts	Total wt. in g.	Fat content p.c.	Iodine no.	Cholesterol p.c.
Unperfused controls	4	0.931	1.43	126	0.107
Perfused 6 hours with Ringer's fluid in oxygen	4	0.662	1.49	117	0.113

however, that perfusion caused no increase in the iodine number of the heart fats. Our figure for the iodine number (126) is higher than the results of Bloor [1926] who found in the ox heart an iodine number of 100 for the phospholipoids and 70 for the fats.

METABOLIC BALANCE SHEET.

The figures shown in our tables permit the construction of an approximate metabolic balance sheet in the case of frogs' hearts perfused in air and in oxygen with Ringer's fluid and with Ringer plasma. This is shown in Table VIII. The figures in the case of air are satisfactory, for the carbohydrate and nitrogen loss are adequate to explain practically the whole of the oxygen consumption and the calculated R.Q. approximates to the R.Q. found. The figures obtained with oxygen are, however, unsatisfactory because the carbohydrate and nitrogen loss together only account for about two-thirds of the oxygen consumption, and the theoretical R.Q. in both cases is higher than the value found experimentally.

An approximate balance could be attained for the two sets of figures obtained with oxygen by assuming that in each case the hearts used

TABLE VIII. Metabolic balance sheet for frogs' hearts.

(All quantities in mg. or c.c. per g. heart per 6 hours.)

	(i) Reducing substances		(ii) Nitrogen		(iii) Respiratory balance calc. from (i) and (ii)		(iv) Respiratory balance found by exp.	
	Oxygen equivalent		Oxygen equivalent		Oxygen used	R.Q.	Oxygen used	R.Q.
	Loss		Loss					
Perfused Ringer's fluid								
(i) In air	1.30	0.97	0.54	3.2	4.2	0.85	4.8	0.90
(ii) In oxygen	0	0	0.59	3.5	3.5	0.80	5.4	0.73
Perfused Ringer plasma								
(i) In air	7.82	5.8	0.27	1.6	7.4	0.98	7.08	0.91
(ii) In oxygen	5.20	3.9	0.20	1.2	5.1	0.96	8.10	0.76

The following factors were used in calculating these figures: 1 mg. $N_2 \equiv 6$ c.c. O_2 (R.Q. 0.8); 1 mg. glucose $\equiv 0.75$ c.c. O_2 (R.Q. 1.0); 1 mg. fat $\equiv 2.02$ c.c. O_2 (R.Q. 0.71).

about 1 mg. of fat ($\equiv 2$ c.c. O_2) per g. Unfortunately the analyses shown in Table VI lend no support to this assumption. We are, therefore, forced to the assumption that in the case of a frog's heart working in pure oxygen about one-third of the oxygen is consumed by some process that does not involve the loss of carbohydrate or fat or involve the excretion of nitrogen, and that this oxygen consumption is associated with only a small excretion of CO_2 .

DISCUSSION.

The authors commenced their investigations on the metabolism of the frog's heart in the expectation that the source of energy used by the heart would prove to be carbohydrate. Moreover the work of Clark and White [1928 b] had shown that any change that altered the mechanical response of the heart produced a parallel change in the oxygen consumption. The first facts established by our investigations were that the frog's heart perfused with Ringer's fluid and working in air used very little carbohydrate and excreted sufficient nitrogen to account for about half the oxygen consumption. Depression of the heart with lack of calcium or alcohol [1932 a] produced a greater proportional decrease in oxygen consumption than in nitrogenous excretion. The present experiments show that stimulation of the heart by perfusion with plasma increased the oxygen consumption, but reduced rather than increased the nitrogenous excretion. Perfusion of the heart with amino-acids produces a moderate increase in nitrogen excretion [1931]. Deprivation of oxygen reduced the nitrogen excretion of the heart to about one-

quarter [1932 *b*, Table V]. (This ratio, in the paper referred to, is stated on p. 329 to be 1 to 25, but this is due to an arithmetical error and the true value is 1 to 4.)

The nitrogenous metabolism of the frog's heart, therefore, remains remarkably constant as long as oxygen is present, but lack of oxygen causes a reduction in the nitrogenous metabolism and a compensatory increase in the carbohydrate metabolism occurs. Even partial oxygen lack causes the heart to convert carbohydrate to lactic acid. Not only does it act on its own carbohydrate, but will also convert glucose added to the perfusion fluid, although the heart when working in air makes no use of carbohydrate added to the perfusion fluid. Hearts in air use about 5 c.c. of oxygen per g. per 6 hours, a quantity which would be consumed by the oxidation of 7 mg. of carbohydrate, whilst the anaerobic heart can produce 34 mg. of lactic acid per g. per 6 hours [1932 *b*, Table V]. The relation between aerobic oxygen consumption and anaerobic lactic acid production in the heart shows, therefore, a considerable resemblance to the relation found in skeletal muscle; but the heart under aerobic conditions uses relatively little carbohydrate.

The substitution of pure oxygen for air does not produce any obvious change in the mechanical activity of the heart. The metabolism is, however, altered considerably. The nitrogen excretion is unchanged, but the carbohydrate metabolism is decreased and there is a remarkable fall in the R.Q., which can only be explained by assuming that about one-third of the oxygen used by the heart is utilized in some process with an R.Q. of 0.7 or even less.

It is surprising that the substitution of oxygen for air causes any change in the metabolism of the isolated frog's heart, because, according to the usually accepted formulae, air ought to provide a more than adequate supply of oxygen. The application of Warburg's formula shows that when a frog's heart in air consumes as much as 2 c.c. of oxygen per g. per hour, an adequate oxygen supply ought to be received when the sheets of muscle do not exceed a thickness of 0.25 mm.

We show in an appended note that the average thickness of the trabeculae of the frog's heart during diastole is about 0.05 mm. and about 0.1 mm. during systole. These figures indicate a large factor of safety, moreover the mechanical performance of the heart suspended in air appears normal and only traces of lactic acid are formed.

Pure oxygen certainly does not exert any toxic effect on the heart and our general impression has been that the hearts survive somewhat better in oxygen than in air. Our results suggest that reducing the

oxygen pressure causes changes in the metabolism before it causes any easily measurable changes in the mechanical activity of the heart. Our results show that in pure oxygen some substance with a low R.Q. is metabolized, but as regards the identity of this substance we can only say that there is no evidence of any fat loss. It is, however, possible that there is a fundamental fallacy in all our calculations regarding the metabolic balance sheet, since these have been based on the assumption that all oxidations in the isolated heart proceed to completion.

In the case of carbohydrate this assumption implies first that there is no appreciable accumulation of non-hexose reducing substances (such as methyl-glyoxal, pyruvic acid, etc.) and secondly that the "lactic acid" found does not include such substances. Case and Cook [1931] have shown that methyl-glyoxal is an intermediate in the breakdown of glucose to lactic acid by minced skeletal muscle of frogs, and hence the assumptions made are of doubtful validity.

In the case of fat there is the possibility that a shortening of the fatty acid chains occurs. Such a shortening would involve usage of oxygen with a very low R.Q. and would not lead to any reduction in the apparent amount of fatty acid present.

Smedley-MacLean and Pearce [1931] have shown that the oxidation of oleic acid involves splitting of the chain at the double bond, a process which actually leads to the production of two fatty acid molecules in place of one.

In view of these and other possible incomplete oxidations our failure to obtain a satisfactory balance sheet by the simplest possible interpretation of the R.Q.'s is not surprising, and we also feel that there are many possible explanations of the increase in reducing substances occasionally observed during perfusion.

CONCLUSIONS.

1. The following changes are observed when isolated frogs' hearts are kept in pure oxygen instead of in air:

- (i) The R.Q. is greatly reduced.
- (ii) The carbohydrate consumption is slightly reduced.
- (iii) The total oxygen consumption is raised about 15 p.c.

2. We have failed to obtain any evidence that isolated frogs' hearts metabolize fat either in oxygen or in air.

3. In the case of hearts working in air, it is possible to work out a metabolic balance sheet, which is in approximate agreement with the measurements of the respiratory exchange and the heart analyses.

4. This cannot be done for hearts working in oxygen. In this case about one-third of the oxygen consumption appears to be utilized in some unknown process, which has a very low R.Q.

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A NOTE ON THE STRUCTURE OF THE FROG'S VENTRICLE.

The frog's ventricle is well known to be a sponge-like structure, but we know of no estimates of its internal surface. This was determined by the following method. Two frogs' ventricles were fixed with Bouinn's Fixative, one was in a contracted state and one was filled with fluid. Dr W. G. Millar kindly cut sections of these, from which microphotographs were prepared. The length of the internal surface was measured by means of a map reader and the values obtained were reduced to the natural scale. The ventricles weighed 0.11 g. In the case of the contracted ventricle the total length of the internal surface shown was 3 cm. The heart was about 7 mm. long, and, if regarded as a cylinder 7 mm. long divided by vertical partitions, the total internal surface will be 21 sq. cm., or about 200 sq. cm. per g. of ventricle. Similar calculations in the case of the dilated ventricle gave a value of 320 sq. cm. per g. of ventricle. Direct observation showed that the average thickness of the trabeculae in the contracted and dilated hearts were 0.1 and 0.06 mm. respectively; this value agrees with the results of the calculations made

above. These figures are minimum values because the trabeculae were assumed to form sheets, whereas in reality they resemble rods more nearly than sheets.

The frog's heart is a much more favourable structure as regards thickness of tissue than even a sartorius of 50 mg., for the latter is about 0.5 mm. thick and has an area of only 40 sq. cm. per c.c. The ventricular muscle has five to ten times as great a surface per unit weight.

These calculations only apply, however, to a ventricle which contracts freely with good diastolic filling and thus irrigates its internal surfaces thoroughly. A strip of ventricle hung up in air has an effective surface of less than 30 sq. cm. per g., which is even lower than the sartorius.

THE GASTRIC FIBRES OF THE VAGUS NERVE.

BY B. A. McSWINEY AND W. R. SPURRELL.

(From the Physiology Department, Leeds.)

EVER since the work of Gaskell and Langley, the vagus nerve has occupied a firmly established position in the parasympathetic nervous system, and amongst its contributions to that system are included the vagal branches to the stomach. In conformity with the general arrangement of neurones in this system, the gastric branches of the vagus are usually considered to be long connector neurones arising in the bulbar nuclei and passing via the vagus trunk to Auerbach's plexus; hence a short effector neurone is relayed to the stomach (Fig. 1, A).

The bulbar roots of the vagus and its trunk above the two vagal ganglia consist almost entirely of medullated fibres, both small and large. Below the ganglia a marked difference is to be found in the composition of the nerve, owing to the appearance of a large number of non-medullated fibres; as the pharyngeal and laryngeal branches leave the parent trunk, they take with them the majority of the medullated fibres so that the proportion of non-medullated increases as the nerve enters the thorax, till finally its abdominal branches consist almost entirely of non-medullated fibres. These changes were recognized by Gaskell, Langley [Schafer, 1900], Ranson [1914] and others, but they made no suggestion that these non-medullated fibres were anything but vagal in their origin, although divergent views were expressed as to how the myelin sheath was lost. Recently Kiss and his colleagues have again drawn attention to this increase in the non-medullated components and have suggested a very different explanation of their presence and origin.

Kiss [1931] has made histological studies of the vagus in man, using an osmic acid technique. He states that non-medullated fibres are not present in the vagus till the lower level of the plexiform ganglion is reached, and he suggests that these fibres are really contributions from the superior cervical ganglion passing to the vagus via the lower anastomotic branches between the sympathetic and vagal ganglia. These anastomotic branches fall into two distinct groups, an upper and a lower; the upper group consists of fine medullated fibres, which Kiss compares

to the white ramus communicans of a spinal nerve; the lower group consists of non-medullated fibres comparable to the grey ramus communicans returning to the spinal nerve. It is, therefore, suggested that the vagus bears the same relationship to the sympathetic as does a typical spinal nerve—fine medullated connector neurones arise in the bulbar nuclei of the vagus, pass via the vagal roots, trunk, plexiform

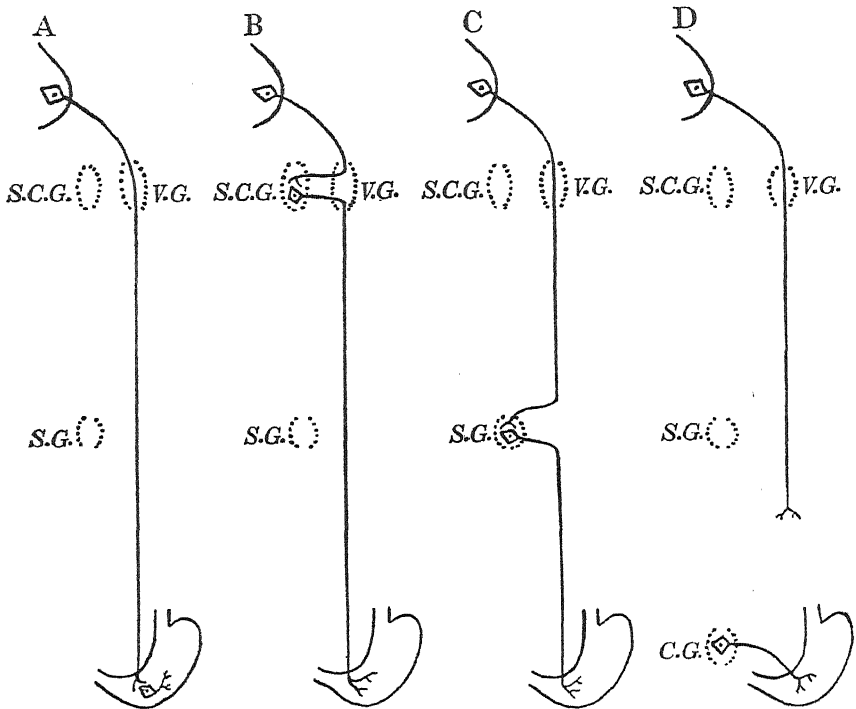


Fig. 1. The possible paths of the gastric fibres of the vagus. A, after Langley; B, C, D, after Kiss. V.G. ganglion trunci vagi; S.C.G. superior cervical ganglion; S.G. stellate ganglion; C.G. coeliac ganglion.

ganglion and upper anastomotic branches to a cell station in the superior cervical ganglion; in the superior cervical ganglion a second neurone begins, rejoins the vagus as a non-medullated fibre in the lower anastomotic branches and passes in the main trunk to its peripheral distribution. As the gastric branches of the vagus are almost entirely non-medullated, he therefore suggests that they have this sympathetic origin. Fig. 1 B gives a schematic representation of this suggested arrangement of neurones.

In further support of this idea, Kiss has made a series of comparative anatomical studies in which he claims that the innervation of the abdominal organs is always sympathetic in origin, though the path taken by the fibres varies in different species. Broadly speaking, the alternative paths fall into two groups, which are represented schematically in Fig. 1 (C, D)—in C the sympathetic contribution arises in the stellate or adjacent ganglia and passes to the thoracic vagus, whereas in D the sympathetic contribution passes direct to the stomach and the vagus, as such, does not reach that organ.

This conception of the arrangement of autonomic fibres in the vagus introduces a fundamentally new principle into the arrangement of the parasympathetic: Kiss goes so far as to say that in the abdomen no such separate system exists. Amongst his conclusions are the following statements: "The so-called parasympathetic influence of the vagus has no anatomical basis in the case of the abdomen. The parasympathetic phenomenon can be only a negative phase of the sympathetic."

Langley, Gad and Josephs [Schafer, 1900] considered that these vagal fibres lost their myelin sheath in their course, probably in the vicinity of the vagal ganglia, while Gaskell, from comparative studies, thought that these fibres represented a relay from a cell station on the vagus trunk.

From further histological studies Kiss [1932] states that he is able to identify a nerve cell which is "sympathetic," *i.e.* it gives rise to a non-myelinated fibre of the sympathetic system. These cells are found, not only in the sympathetic ganglia, but also in cranial and spinal ganglia, and they are therefore credited with supplying sympathetic elements within the cranial and spinal nerves. On these grounds he again makes the statement that: "There is no morphological foundation for the supposition of a cranial parasympathetic system."

Apparently Kiss takes the view that all the nervously controlled phenomena termed "parasympathetic" result from impulses passing down a system of non-myelinated fibres which are essentially "sympathetic" and arise from a certain type of cell within a sympathetic ganglion or in one of the cranial ganglia.

In order to test this theory we have performed experiments to determine the functional pathway whereby the vagus influences the stomach, and have tried to establish the source of the non-medullated components of the nerve. The animals employed have been cats. In these animals the histological differences in the vagus trunk are of a similar nature to those described in man—there are a few non-medullated

fibres in the vagal roots, but the number is markedly increased below the ganglion of the trunk. As the vagus does reach the stomach in the cat, condition D (Fig. 1) does not arise, and we have devoted our attention to the three other possibilities, A, B and C.

EFFECTS OF STIMULATION OF INTRACRANIAL VAGUS.

If the functional path of the gastric fibres is through the superior cervical ganglion, as suggested in B (Fig. 1), then removal of the superior cervical ganglion, and stimulation of the vagus above its ganglia should produce no effect upon the stomach. As the extracranial portion of the vagus above its ganglia is too short and inaccessible for satisfactory stimulation, we therefore proceeded to study the effect of stimulation of the intracranial portion of the vagus and the following technique was developed.

Method.

The animals were anaesthetized with ether, the carotids tied, and decerebration carried out by means of a trephine and scoop. The animals were then given a little more ether and the spinal cord was divided at the level of the axis vertebra; this left the animal with the lower part of the brain stem intact, but isolated from the spinal cord. All the operative procedures involved in the decerebration and spinalization were carried out with the cautery to minimize hæmorrhage. The animal was then allowed to recover for about 30 min.

The abdomen was opened and the pylorus was tied off; the œsophagus was opened in the neck and a stomach tube passed through the cardia and tied in position. The stomach was then washed out with saline and 60 c.c. of warm meat extract were introduced and the animal allowed a further 30 min. rest.

The cerebellum and bulb were now completely exposed by removing the overlying bone. The stump of the cervical cord was lifted up and gently mobilized until the bulbar roots of the vagus were clearly visible—the roots were easily found by following the spinal accessory nerve as it ran up along the lateral border of the bulb to join the lower vagal root. When the vagal roots were identified, two longitudinal cuts were made in the bulb, and its middle third removed—this allowed considerable freeing of the roots and avoided any risk of current spread to the opposite side. At this point, there was frequently troublesome oozing from the divided lateral sinus and bone, but this was controlled by light packing with wool. With this exposure it was possible to identify five bundles of

fibres going to form the vagus trunk—they could either be stimulated together or gently separated to permit of stimulation of individual bundles. There was no difficulty in identifying the spinal portion of the spinal accessory; it was much whiter and firmer than the vagal roots, which were pinkish, and soft in appearance, suggesting the absence of any quantity of supporting fibrous tissue.

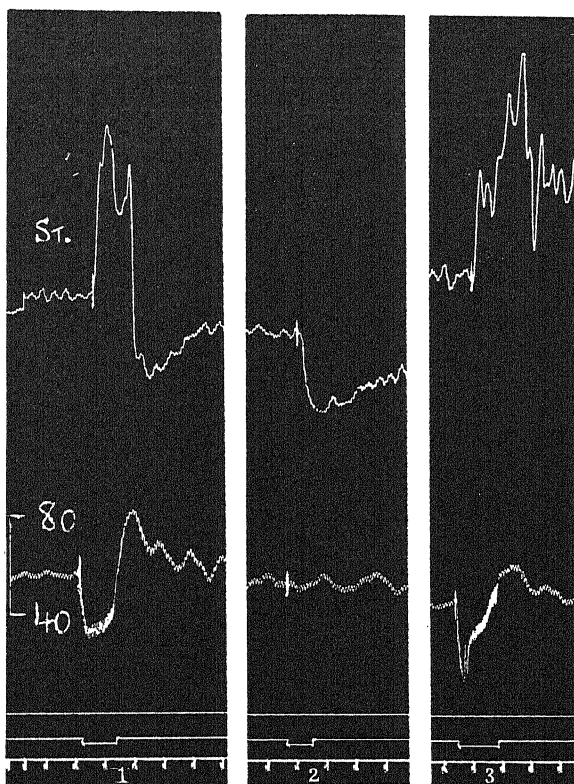


Fig. 2. Gastric response to stimulation of the intracranial roots of the vagus. Cat 12. (1) Motor response—right vagal roots; coil 12, freq. 10: (2) inhibitor response—right vagal roots; coil 10, freq. 60: (3) motor response—cervical trunk of right vagus; coil 8, freq. 20. Time intervals 30 sec.

When the bulbar exposure was completed, the stomach-tube was connected to a recording manometer and the right carotid was cannulated. Stimulation of the intracranial vagus was then carried out either upon the whole nerve or upon individual roots. Towards the end of the

experiment the cervical vagus on the same side was stimulated for comparison of results.

When the results of intracranial stimulation had been established the same procedure was repeated in animals in which the superior cervical ganglion had been removed.

Experimental results.

Stimulation of the intracranial vagus produced exactly similar effects upon the stomach as did stimulation of the cervical trunk—motor or or

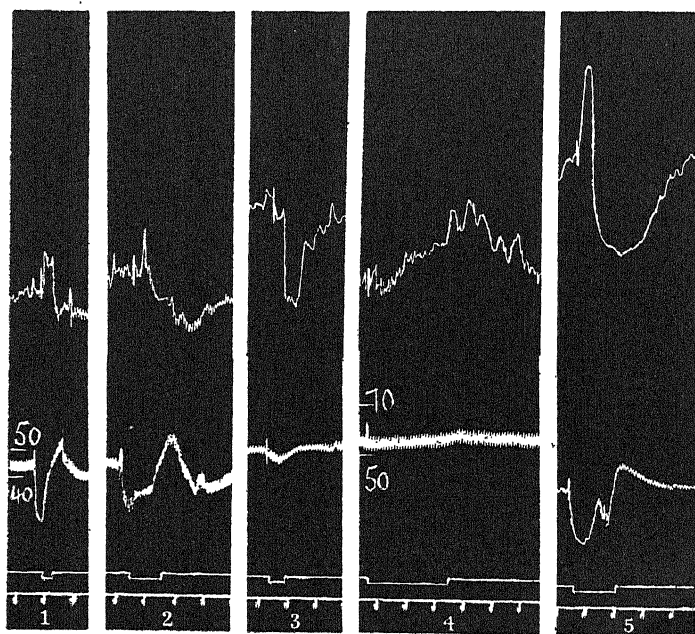


Fig. 3. Gastric response to stimulation of the intracranial roots of the right vagus after removal of the right superior cervical ganglion. (1-4) Stimulation of right vagal roots. Note the progressive diminution in the concomitant cardio-vascular response. (5) Stimulation of cervical trunk of right vagus after (4). Time intervals 30 sec.

inhibitor effects were recorded depending upon variations in the intensity of stimulation, tone of the stomach and the animal (Fig. 2). Selective stimulation of individual roots produced results which only varied quantitatively from those of the whole nerve, *i.e.* it was never possible to identify any particular bundle of fibres as being associated with any particular effect, motor or inhibitor.

The cardiac response to stimulation of the intracranial vagus showed very definite characteristics. At the commencement of the experiment even weak stimulation produced a marked and prolonged fall in blood pressure. As the experiment proceeded, the cardiac fibres became steadily less sensitive, till finally no cardiac effect could be elicited. That this loss of sensitivity of the cardiac fibres was purely a local condition was proved by the ease with which cardiac effects could be elicited via the cervical vagus long after the intracranial portion of the nerve was quite ineffective.

The disappearance of cardiac response did not affect the gastric response, showing that the latter was not dependent upon circulatory changes (Fig. 3).

The gastric response to stimulation of the intracranial roots of the vagus was quite unaffected by removal of the superior cervical ganglion (Fig. 3).

THE STELLATE GANGLION AND THE VAGUS.

Possibility C (Fig. 1) suggests that the gastric branches of the vagus might be derived from the stellate ganglion; if this were so, then removal of the stellate ganglion should render stimulation of the cervical portion of the vagus ineffective. Experiments were performed to test this point.

Method.

Decapitate cats were used. The stellate ganglion was exposed on one side and temporarily covered with a pad moistened with saline. The abdomen was then opened and the pylorus tied—a stomach tube was inserted through the oesophagus and connected to a water manometer. The right carotid was cannulated and the vagus nerve of the side corresponding to the exposed stellate ganglion was exposed and divided high up in the neck. Records of the gastric response to stimulation of the cervical vagus were then made over a wide range of intensities and rates. The stellate ganglion was then excised and a similar range of stimulation was repeated on the cervical trunk of the vagus.

Experimental results.

No difference could be detected between the gastric responses with and without the stellate ganglion.

THE ORIGIN OF THE NON-MYELINATED FIBRES OF THE VAGUS.

Having shown that the motor supply of non-medullated fibres to the stomach was not sympathetic in origin, it was necessary to enquire further into their mode of arrival. Two possibilities exist—they may

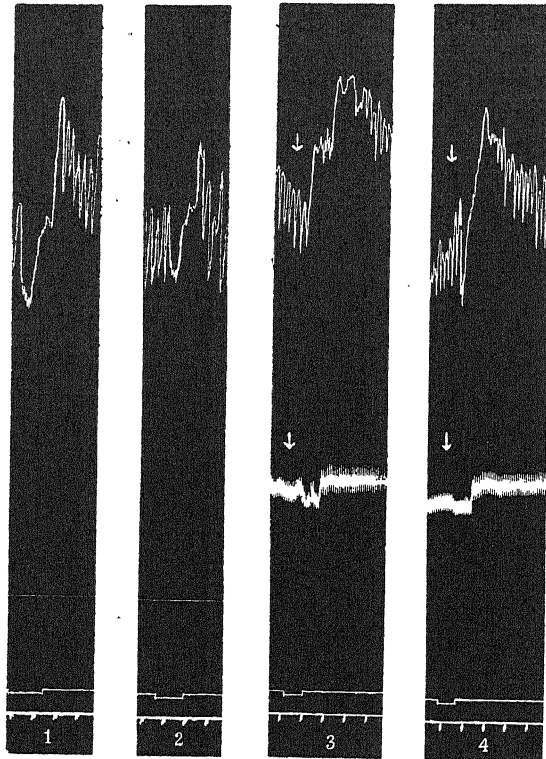


Fig. 4. Gastric response to stimulation of the cervical trunk of the vagus before and after removal of the corresponding stellate ganglion. Cat S 2. (1) Right vagus, ganglion intact; coil 4, freq. 24; (2) right vagus, ganglion removed; coil 4, freq. 24. Cat S 4. (3) Left vagus, ganglion intact; coil 3, freq. 60; (4) left vagus, ganglion removed; coil 3, freq. 60. Time intervals 30 sec.

represent connector neurones arising in the bulb and losing their medullary sheaths en route (Fig. 5, A), or they may represent a fresh neurone arising within one of the ganglia of the vagal trunk (Fig. 5, B). The former view was supported by Langley, Gad and Josephs [Schafer, 1900] but comparative studies suggested the latter explana-

tion to Gaskell. In the cat, longitudinal sections through the ganglion of the trunk show that the change to non-myelinated fibres takes place at that level.

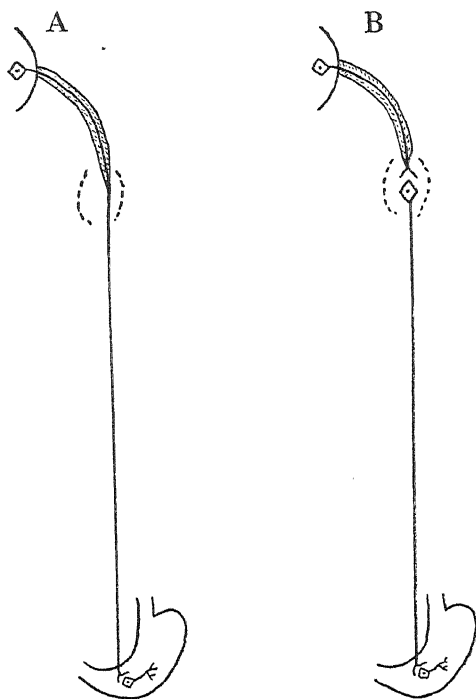


Fig. 5.

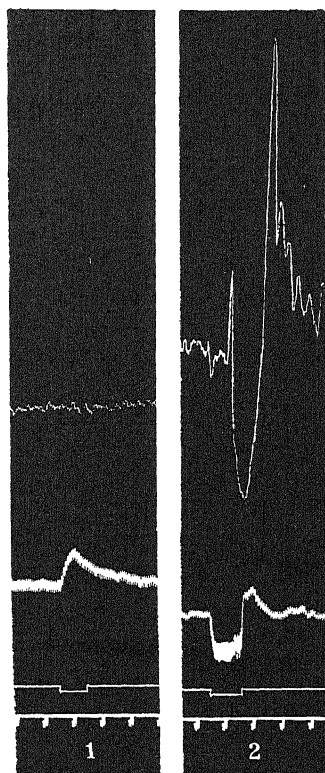


Fig. 6.

Fig. 5. Possible origins of the non-myelinated fibres. A, after Langley, Gad and Josephs; B, after Gaskell and Kiss.

Fig. 6. Effect of section and degeneration of vagus. Cat V 4. Right vagus divided above vagal ganglion 27 days previously. (1) Stimulation of cervical trunk of right vagus; coil 4, freq. 60: (2) stimulation of cervical trunk of left vagus; coil 4, freq. 60. Time intervals 30 sec.

Method.

In a series of cats the right vagus was divided immediately on emergence from the skull; the animals were allowed to recover and 10 to 28 days later the cervical vagus was stimulated and its effect on the stomach recorded.

Experimental results.

The cervical trunk of the vagus on the side of section was decidedly softer and more translucent than on the intact side. Stimulation of the sectioned vagus produced no motor or inhibitor effects upon the stomach in any way comparable to those obtained by stimulation of the intact nerve; the only change recorded was an occasional slight diminution in the amplitude of gastric contraction, most noticeable in those cases where a striking alteration in the circulation was produced: we are inclined to attribute this slight alteration in gastric response to the coincident vascular change.

The vascular response to stimulation of the sectioned vagus was in all cases a definite rise in blood-pressure; there was a latent period of 6 to 8 sec. and little, if any, acceleration of the heart rate. In all animals the right vagus was cut and the results correspond with the preponderance of pressor responses described by Morgan and Goland [1932] in the right vagus of the dog.

DISCUSSION.

The fact that typical gastric responses to vagal stimulation can be obtained in cats deprived of the superior cervical ganglion or stellate ganglion indicates that the nervous pathway involved does not pass through these ganglia. The functional path of the gastric fibres of the vagus is confined to that nerve and derives no essential contribution from the sympathetic.

Section of the vagus trunk above the vagal ganglia results in the degeneration of the fibres which conduct the impulses to the stomach. Therefore no cell station is present in these ganglia, and it would appear that only one neurone is concerned in the conduction between the bulb and the stomach. Histological examination clearly indicates that the bulk of these fibres leave the medulla with medullary sheaths and reach the stomach as non-myelinated fibres; the site of loss of the myelin sheath is in the vicinity of the vagal ganglia.

As far as the efferent gastric fibres of the vagus are concerned, these experiments lend no support to Kiss's theories as to the relationship between the vagus and the sympathetic. Whatever may be the function of his "sympathetic" cells in the vagal ganglia, they are not the cells of origin of the non-myelinated efferent fibres to the stomach.

It has been suggested that the cervical sympathetic may send contributions via the superior cervical ganglion to the vagus, and that these

fibres are responsible for some of the gastric effects elicited by stimulation of the cervical vagus. These experiments show that stimulation of the intracranial roots of the vagus will produce the same range of gastric response as does the cervical vagus, a finding which disposes of any essential contributions from the cervical sympathetic.

Attention is drawn to the apparent difference between the cardiac and gastric fibres in the vagal roots. The cardiac branches are exceedingly sensitive at the commencement of the experiment, but this sensitivity rapidly diminishes till no cardiac effect can be elicited although the gastric response is undiminished (Fig. 3). This high degree of primary sensitivity with rapid subsequent fall suggests that the cardiac fibres have little protective covering in the vagal roots: thus they are readily accessible to stimulation but soon suffer damage thereby.

It has been suggested that the variations in gastric response to vagal stimulation are dependent upon or profoundly influenced by concomitant vascular changes. The fact that the intracranial cardiac roots become insensitive to stimulation, whilst the gastric fibres remain intact, enables one to separate circulatory and gastric effects; the response of the stomach appears to be little affected by the presence or absence of simultaneous circulatory changes (Fig. 3).

Stimulation of individual bundles of the vagal roots produced results which only varied quantitatively from those of the whole nerve. No evidence was obtained for the existence of special groups of motor or inhibitor fibres.

The arrangement of the gastric fibres of the vagus appear to conform to Langley's original conception of the parasympathetic system. A myelinated fibre leaves the bulb and passes via the vagus trunk to the stomach; in its course it loses its myelin sheath but has no cell station in the vagal ganglia.

SUMMARY.

1. Stimulation of the bulbar roots of the vagus produces types of gastric response similar to those elicited from the cervical vagus.
2. Stimulation of individual roots produces no evidence of different types of nerve fibre responsible for motor or inhibitor effects on the stomach.
3. Vascular changes are not responsible for the various types of gastric response.
4. The functional path of the gastric fibres of the vagus is confined to that nerve, and has no essential connection with the sympathetic.

5. There is no cell station for the gastric fibres in the vagal ganglia. Myelinated fibres leave the bulb and pass directly to the stomach, losing their myelin sheaths en route.

One of us, W. R. Spurrell, is indebted to the Medical Research Council for a personal grant.

The expenses of this investigation have been defrayed in part by a grant from the Government Grant Committee of the Royal Society.

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PROCEEDINGS
OF THE
PHYSIOLOGICAL SOCIETY,
October 22, 1932.

The fallacies of indirect calorimetry. By T. W. ADAMS
and E. P. POULTON.

Insulin raises the R. Q. in depancreatized animals and so increases the utilization of carbohydrate; but in depancreatized animals the R. Q. may be fairly high—up to about 0.80, so that the power to utilize carbohydrate is not lost. These facts, apparently contradictory, led us to question the basis of indirect calorimetry, viz. that the level of the non-protein R. Q. indicates the proportion of c. to f. oxidized, the value being based solely on the chemical composition and the heat combustion of c. and f. [Zuntz and Schumburg]. Those published experiments were examined in which the calories, O_2 and CO_2 , were simultaneously determined over periods of 1, 2 and 3 hours. In each case non-protein values were obtained and the proportions between indirect and direct calorimetry calculated; these quantities were then plotted against the R. Q. In the figures the heat measured is taken as 100 p.c. and is represented by the horizontal line at zero; the ordinates for the top part of the diagram represent the percentage differences between the calculated and measured heats. The proof that it is permissible to use the Zuntz-Schumburg figures will depend on the black dots lying evenly distributed above and below the horizontal line throughout the range of R. Q.s. This is obviously not the case (Figs. 1, 2 and 3), and a fall in the percentage differences as the quotient rises has been found in all the published experiments on fasting subjects.

There is a slight difference in the slope of the results for A. H. M. and A. L. L., and the mean slope for these two men is the same as that drawn in Fig. 3 and it cuts the zero line at R. Q. 0.785, indicating a combustion of c. to f. in the ratio of 1 : 1.36. Hence though it is necessary to give up Zuntz's theory the provisional hypothesis may be advanced that c. and f. are always burnt in this constant proportion and at other R. Q.s additional metabolic changes take place which influence the heat produced. In this connection it is interesting that when the fast is prolonged the point of intersection of the slope with the zero line shifts to the left (Fig. 3) indicating that the proportion of c. to f. burnt has changed for S. A. B. in the course of a 3 days' fast from 1 : 1.14 to 1 : 1.34; this is perfectly

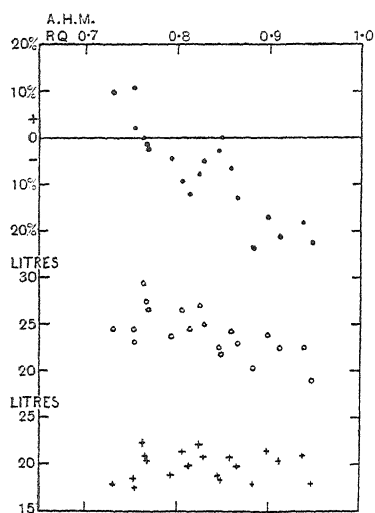


Fig. 1.

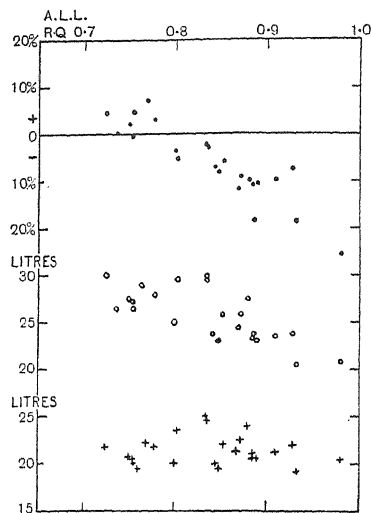


Fig. 2.

Comparison of indirect and direct calorimetry; \circ oxygen; $+$ CO_2 , two hourly determinations during first day of fasting. Subject at rest. *Carnegie Institution Publ. 261.*

Figs. 1 and 2. Periods of 2 hours each during day.

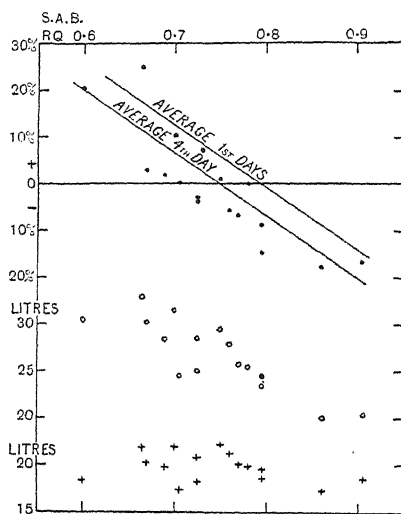


Fig. 3.

Subject not at complete rest. *Carnegie Institution Publ. 77.*

Fig. 3. Periods of 2 hours each, from 9 a.m.—11 p.m.

intelligible considering that there is less c. now available in the body. Fortunately in most determinations of the basal metabolism the R. Q. is in the neighbourhood of 0.80 so that the Zuntz-Schumburg figures usually give correct results.

The thermochemical equations for the conversion of c. to f. without the formation of intermediate products do not account for the difference between the calculated and measured heats, and so it is probable that temporary partial metabolic changes are taking place.

In the figures the O_2 and CO_2 values have also been entered, and it is of particular interest to note that while the CO_2 remains constant the O_2 intake diminishes as the R. Q. increases. This is the converse of what happens when sugar is taken.

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On the mode of action of vasomotor nerves. By W. A. BAIN. (*Department of Physiology, University of Edinburgh.*)

That certain nerves produce their effects by the production or liberation at their endings of specific chemical substances is now fairly well established. This has been directly demonstrated by Loewi [1921] and numerous others in the case of the cardiac nerves of the frog, by Rijlant [1927] for the cardiac nerves of mammals, by Finkleman [1930] and by Cannon and Bacq [1931] for the sympathetic supply to mammalian plain muscle. Indirect proof that vasodilator nerves act likewise has recently been given by Dale and Gaddum [1930].

An attempt has been made to obtain direct evidence in favour of the view that vasomotor nerves act by the production or liberation of specific substances at their terminations. In these experiments the tongue (*in situ*) of the dog, perfused with Dale's solution by means of a Dale-Schuster pump, was used. The fluid entered the tongue *via* the lingual artery and on leaving *via* the vein was immediately led to an intestine bath in which was suspended a short piece of rabbit intestine as a test tissue. The perfusion pressure was recorded by a mercury manometer connected with the output tube of the pump. In order to ascertain whether the rate of flow through the tongue varied with changes in calibre of the tongue vessels, the outflow from the tongue was recorded by an electric tilter operating from the overflow of the intestine bath. By this means it was easy to show that the effects obtained on the isolated

intestine by stimulation of the vasomotor nerves of the tongue were not due to changes of temperature in the intestine bath consequent upon a changed rate of flow through the tongue vessels.

It has been shown that: (1) stimulation of the peripheral end of the lingual nerve confers upon the perfusing fluid properties which render it excitatory to an isolated length of rabbit intestine, causing an increase in amplitude of the contractions and usually also an increase in tone of the muscle. The average increase in amplitude over the normal varies in different experiments between 20 and 80 p.c.; (2) stimulation of the peripheral end of the vagosympathetic trunk confers upon the perfusing fluid properties which render it inhibitory to an isolated length of rabbit intestine, the contractions becoming diminished in amplitude and the tone usually becoming slightly reduced. The average decrease in amplitude below normal varies between about 20 and 40 p.c. in different experiments.

It is concluded that these effects on the isolated intestine strip are due to the humoral transmission of specific substances produced or liberated by the agency of the nerves supplying the tongue vessels, and that it is by the production of these substances and their subsequent action upon the muscular tissue of the vessels that the vasomotor nerves of the tongue produce their effects. The fact that definitely positive results have not been obtained in cases where blood was present in the perfusing fluid supports the view that, under normal circumstances, the neuromimetic substances are either not passed into, or are rapidly destroyed by the blood. It is therefore suggested that normally these neuromimetic substances produce their effect locally, at the site of production, and that any humoral transmission to other sites is probably incidental and unimportant.

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The action of œstrin on the isolated uterus. By G. F. MARRIAN and W. H. NEWTON.

For these experiments, strips of uterine muscle have been taken from guinea-pigs and suspended in the solution recommended by Burn and Dale [1922]. To the solution have been added oxytocin and pure crystalline œstrin. Both the trihydroxy- and ketohydroxy- varieties of

the latter have been used, dissolved in either absolute alcohol or sodium hydroxide solution. When alkali has been employed, an equivalent amount of hydrochloric acid has been added simultaneously, and in all cases control experiments have been performed, the solvent being added without the oestrin. 5 p.c. carbon dioxide in oxygen has been bubbled through the bath instead of pure oxygen, so as to complete the bicarbonate buffer system.

Fig. 1 shows eight consecutive responses of a virgin uterus to the same dose of oxytocin (0.025 unit), the solution being changed after each contraction and the interval between each being the same (15 minutes). Before the first two doses there was no preliminary treatment; $2\frac{1}{2}$ minutes

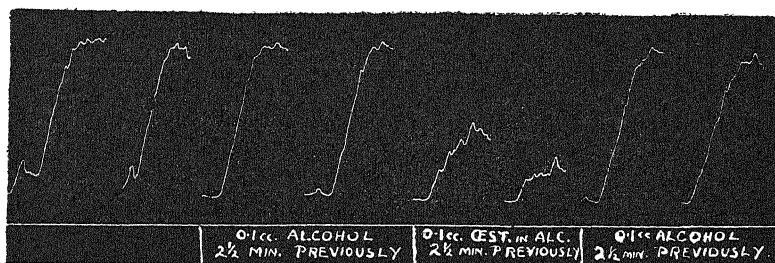


Fig. 1. Virgin guinea-pig, 220 gm. Eight consecutive doses of 0.025 unit oxytocin at 15 min. intervals.

before each of those of the second and fourth pair, 0.1 c.c. of absolute alcohol was added to the bath; $2\frac{1}{2}$ minutes before each of the third pair, was added 0.21 mg. of ketohydroxy-oestrin dissolved in 0.1 c.c. of absolute alcohol.

The same result has been obtained for both the trihydroxy- and ketohydroxy- compounds, using for the test, instead of the virgin uterus, the uterus three months after oöphorectomy.

The second half of the tracing in Fig. 2 shows the reaction of the pregnant uterus to oxytocin (0.025 unit, left in the bath), and the subsequent effect of 0.2 mg. of ketohydroxy-oestrin dissolved in sodium hydroxide solution, the equivalent of acid being added simultaneously. The first half of the tracing shows the effect of equal amounts of alkali and acid under the same conditions. Exactly the same effect is seen when alcohol is used as a solvent; it may be summarized as a more rapid relaxation of the strip from each contraction, a return to a much greater resting length, and a speeding up of the rhythm.

One conclusion may be drawn from the combined results of the three sets of experiments (virgin uterus, involuted uterus and pregnant uterus), namely, that œstrin counteracts the tendency of oxytocin to produce any sort of sustained contraction of the uterus. Its effect on rhythmic contraction is not so clear. Fig. 2 would suggest that the combination of oxytocin with œstrin is ideal for labour, and this may be so. But the

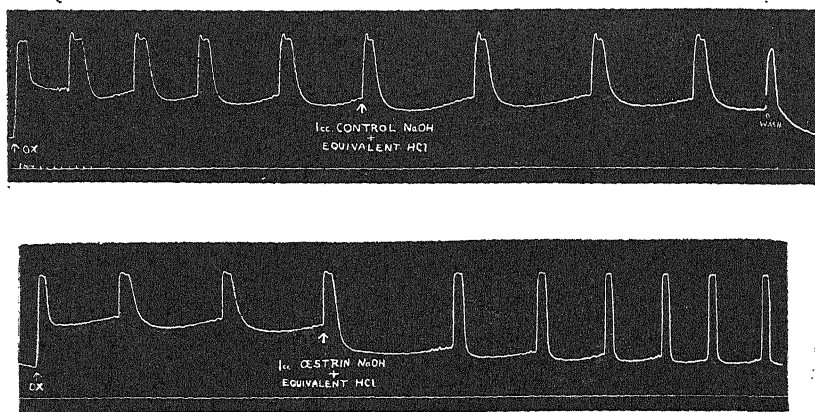


Fig. 2. Pregnant guinea-pig, 600 gm. Nearly at full term; length of foetus 10 cm.
OX \equiv 0.025 unit oxytocin. The two halves of the tracing are continuous.

mature non-pregnant uterus, and the uterus of early pregnancy, show maximal rhythmic contractions in the absence of oxytocin, and the action of œstrin upon these is depressor both as regards their height and frequency. The possibility must be considered, therefore, that the heightened rhythm in Fig. 2 is in some way due to the more relaxed state of the muscle rather than to the presence of œstrin.¹ We have been handicapped throughout by the insolubility of œstrin in physiologically inert solvents, and the consequent impossibility of adding more than a small quantity to the bath (capacity 60 c.c.).

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Gas tensions in the gall bladder. By J. ARGYLL CAMPBELL. (National Institute for Medical Research.)

There are no data, so far as I know, regarding the normal carbon dioxide and oxygen tensions in the gall bladder. Previously attempts were made to estimate the tensions in bile by estimations of the volume

¹ Confirmed later.

p.c. of gases removable by gas pump from bile. The literature on this point was reviewed recently [Campbell, 1931], and no definite conclusions could be drawn.

In the present research the animals were anaesthetized (A.C.E. and amytal), the abdomen opened and a gas mixture, containing oxygen, carbon dioxide and nitrogen in varying tensions (see Table I), was injected carefully into the gall bladder in its normal condition and containing bile. Sufficient gas was injected to distend the bladder moderately, a fine hypodermic needle and rubber bag being employed. No ligatures were applied to the ducts, etc., but the abdomen was closed. The gas was left in the gall bladder for various periods, and then samples were withdrawn carefully by means of a similar needle and a gas sample tube or 1 c.c. syringe and then analysed by the modified Krogh method [Campbell, 1932 *a*] or by the Haldane method. The former method was employed when only a bubble (up to 0.15 c.c.) could be obtained for analysis; the Haldane method was used when 1 c.c. or more of gas was available for analysis, the gas being then mixed with a known amount of nitrogen in the 10 c.c. apparatus.

From the details shown in Table I and after allowing for the effects of

TABLE I. Gas tensions in gall bladder.

Date	Animal	Tensions mm. Hg in gas injected		Tensions mm. Hg in gall bladder		Time gas was left in gall bladder (hours)	Method of gas analysis
		CO ₂	O ₂	CO ₂	O ₂		
30. vi. 32	Rabbit	0	43	{ 47 49 56	{ 42 40 45	{ 2.50 3.75 4.75	Krogh, modified
6. vii. 32	"	0	7	{ 49	{ 42	{ 1.50	
13. vii. 32	"	0	19	{ 63	{ 18	{ 3.00	
15. vii. 32	"	0	37	{ 38	{ 29	{ 4.00	"
19. vii. 32	"	0	4	{ 38	{ 28	{ 4.00	
20. vii. 32	"	0	5	{ 31 36 35	{ 27 32 28	{ 3.00 4.50 2.00	
5. vii. 32	Cat	0	4	{ 41	{ 33	{ 3.00	"
28. vii. 32	"	40	26	{ 45	{ 45	{ 3.00	
28. ix. 32	"	0	4	{ 35 56 53	{ 28 19 7	{ 1.66 4.00 2.00	
29. ix. 32	"	0	4	{ 40 42	{ 17 7	{ 3.00 1.50	"
30. ix. 32	Dog	0	4	{ 47 48	{ 13 11	{ 3.83 5.50	

anaesthetics [Campbell, 1932 *b*] it is concluded that the normal carbon dioxide tension in the gall bladder of the rabbit, cat and dog varies between 35 and 60 mm. Hg, and the oxygen tension between 17 and 45 mm. Hg. There is a suspicion that when the oxygen tensions fell below

20 mm. Hg. the circulation in the liver was being interfered with by the position of the animals, which were tied down on their backs. The circulation in these cases did not appear to be active either in the vessels of the stomach or in those of the liver. The supine position is not a normal position for these animals. Nevertheless, there is a general agreement between these results and those published for gas tensions in other tissues of the body [Campbell, 1931, 1932 *b*, *c*]. Many of the latter results were obtained from animals in the normal prone position.

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Hypertrophy of the heart in acclimatization to chronic carbon monoxide poisoning. By J. ARGYLL CAMPBELL. (*National Institute for Medical Research.*)

In the course of the post-mortem examinations of tar-cancer mice, some of which had been exposed for about 9 months to chronic carbon monoxide poisoning and others for the same period to oxygen at high pressure (60 p.c. atmos.) in the air, it was observed that the average weight of the hearts of the mice exposed to carbon monoxide was much greater than that of the mice exposed to high oxygen and also than that of the control mice (see Table I). Only one of the controls and two of the mice exposed to high oxygen possessed hearts weighing more than 0.235 gm. Of the 19 mice exposed to carbon monoxide, 14 possessed hearts weighing between 0.235 and 0.340 g.; in 4 others the weights were between 0.200 and 0.235 g.; 0.435 g. was the weight of the heart in the remaining mouse. The mice were gradually acclimatized to the carbon

TABLE I. Hearts of all the mice surviving to the end of experiment.

Condition of mice	No. of mice	Heart weights (g.)	
		Average	Limits
Exposed to CO	19	0.277	0.435 - 0.200
Exposed to high O ₂	33	0.182	0.260 - 0.110
Controls	32	0.165	0.260 - 0.118

monoxide, so that on the 1st, 5th, 10th, 20th, 24th, 27th, 39th and 46th days the percentages of carbon monoxide in the air breathed were 0.04, 0.06, 0.09, 0.10, 0.13, 0.17, 0.19 and 0.24 respectively. From the 46th day till the 269th day or end of the experiment the carbon monoxide was

maintained at 0.24 p.c. and the surviving mice were evidently acclimatized to this amount as their body weights were as well maintained as those of the other two groups. The respiration chambers employed have been described elsewhere [Campbell, 1932].

The carbon monoxide, on the whole, retarded the development of tar warts and cancer, and this aspect of the experiment will be considered in another paper. Taking ten mice of each group which were equally affected by the tar—judging from the extent of the scar formation, etc.—we have the following weights for the hearts at the end of the experiment (Table II).

TABLE II. Hearts of ten tarred mice of each group compared with those of ten normal mice of similar body weight (28 g.).

Condition of mice	Heart weights (g.)	
	Average	Limits
Exposed to CO	0.282	0.435 - 0.210
Exposed to high O ₂	0.195	0.260 - 0.150
Tarred controls	0.153	0.190 - 0.130
Normal controls	0.149	0.170 - 0.130

We have also data from a few untarred mice which were included in the same long experiment; weights of the hearts are given in Table III, the body weights (28 g.) and diet of each group being similar.

TABLE III.

Condition of mice	No. of mice	Heart weights (g.)	
		Average	Limits
Exposed to CO	2	0.260	—
Exposed to high O ₂	4	0.177	0.190 - 0.165
Controls	4	0.182	0.207 - 0.155

In all cases the entire hearts were weighed moist immediately after the animals were killed, the cavities being slit open to remove blood and blood clots.

The hypertrophy of the heart muscle probably follows the increase in viscosity of the blood owing to increase in red corpuscles [Naismith and Graham, 1906; Campbell, 1929, 1932].

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An isolated mammalian heart preparation capable of performing work for prolonged periods. By I. DE BURGH DALY and W. V. THORPE. (*Preliminary communication.*)

It has been reported [Daly, 1927; Verney, 1927] that an isolated dog's heart preparation performing work rapidly becomes hypodynamic in spite of good oxygenation of the defibrinated blood by some form of Hooker-Drinker apparatus. The onset of cardiac failure generally occurs within 20-60 minutes and is in marked contrast to the conditions found in the heart-lung preparation which, if not overworked, may continue to function well for 5 hours.

The following were considered possible causes of the early cardiac failure in the isolated heart preparation: (1) the removal of any nervous control arising in the lungs; (2) damage to the heart due to operative interference; (3) loss of water from the blood due to evaporation in the oxygenator; (4) physico-chemical changes in the blood due to its passage through the oxygenator with the formation of substances causing the cardiac failure; (5) the addition to or removal from the blood by the lungs of one or more substances necessary for the proper functioning of the heart. We have shown that the factors (1) to (3) are not responsible for the early cardiac failure, although it is true that the heart lasts longer if the pericardium is not opened except for a small slit opposite to the left auricular appendage for insertion of the cannula. Our experiments indicate that an essential condition is extensive blood filtration before and during the experiment through finer cambric filters ($50 \times 100\mu$ mesh measured dry) than those usually employed for heart-lung preparations ($100 \times 200\mu$). The blood should be filtered through four or five filters immediately after defibrination and just before use, and two filters kept in the circuit during the experiment. Anrep and Häusler [1928] stress the importance of thorough blood filtration and detoxication in their experiments on the perfusion of a coronary artery in the heart-lung preparation. We are able to confirm the importance of these factors relative to the isolated heart doing work and the perfused heart performing no work; moreover, we gain the impression that circulation of all the blood through the heart-lung preparation before switching over to the isolated heart, largely prevents the clogging of the filters during the experiment. With these modifications of the usual technique we have been able to keep the isolated heart of a dog performing work over 5 hours (pericardium closed) in addition to 1 hour during which the heart-lung preparation was running. It is clear that the isolated heart requires more perfect filtration of the blood than the heart-lung preparation, and the

reason may be that the rôle of the lungs as a mechanical filter of blood is lacking, or that changes in the physico-chemical state of the blood in the absence of the lungs and in the presence of the oxygenator may lead to the formation of particles injurious to the heart.

The problem of the oxygenation of the blood was solved by building an apparatus which is essentially three Hooker-Drinker oxygenators arranged concentrically. This apparatus, which has a surface area of 1.1 sq. m., can maintain blood at 90-100 p.c. saturation during the experiment.

In two experiments the oxygen consumption per g. of heart per hour varied between 1.4 and 5.6 c.c. These figures agree fairly well with those of Lovatt Evans [1912] who obtained corresponding values ranging from 3.5 to 6.0 c.c. For comparison of the oxygen consumption of the heart at different stages in the experiment, we have estimated the oxygen consumption per g. of heart per kg. work per hour; this value is found to increase as the experiment progresses.

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A simple method of appreciating the muscular sense.

By D. F. FRASER-HARRIS.

One lies supine full-length in a large bath of water. Allow one leg, abducted and slightly flexed at the knee, to float comfortably on the surface of the water. Note the minimal muscular sensations from this resting limb (physiological zero).

Now very slowly begin to immerse the leg, still abducted and flexed, and note the sense of effort (resistance) due to the resistance of the water.

Let the leg return to the surface and once more float at rest, when one perceives a distinct and immediate diminution of the sense of effort.

Finally keeping the leg rigid as one segment from the hip joint and still abducted and flexed, raise it slowly into the air against gravity.

Note the very marked increase of muscular sensation (sensation of weight) which is maximal at the moment of the limb leaving the water and becomes less and less massive as the vertical is approached.

Exactly similar but less massive muscular sensations may be experienced by lowering and raising the arm in one segment from the shoulder joint.

This method, requiring no apparatus, is more "natural" than that of faradizing the muscles (say) of the arm through the skin. In the method just described, the muscular sensations are not complicated by the unpleasant pricking cutaneous sensations produced by the faradic currents traversing the skin.

PROCEEDINGS

OF THE

PHYSIOLOGICAL SOCIETY,

November 19, 1932.

The rate of glomerular filtration in the perfused kidney.

By A. HEMINGWAY. (*Physiology Institute, Cardiff.*)

The amount and rate of glomerular filtration occurring in the kidney of the pump-lung-kidney preparation has been studied according to the creatinine elimination method of Rehberg [1926].

Creatinine is added to the perfusing blood in the proportion of 10 mg. of creatinine dissolved in 1 c.c. of saline for each 100 c.c. of blood. After allowing time for thorough mixing, simultaneous readings are made of blood plasma creatinine concentration (C_b), urine creatinine concentration (C_u), rate of urine output and rate of renal blood flow.

The concentration ratio of creatinine between blood and urine ($\frac{C_u}{C_b}$) is calculated, and, multiplied by the rate of urine formation (U), gives the rate of glomerular filtration (F). The amount of fluid reabsorbed in the tubules (R) = $F - U$.

TABLE I. Rate of glomerular filtration in the perfused kidney.

Exp.	Wt. of kidney g.	U	C_u mg./ 100 c.c.	C_b mg./ 100 c.c.	F	R	$\frac{R}{F} \times 100$	Blood flow
1	23.4	0.74	130.0	16.30	5.92	5.18	87	—
2	22.0	0.72	14.3	3.08	3.31	2.59	76	134
3	24.4	0.90	59.0	4.20	12.60	11.70	93	116
4	32.2	1.06	42.0	6.25	7.10	6.00	85	71

All rates of flow are given in c.c./min.

Table I shows the rates which have been measured in some preliminary experiments. The maximum rate of glomerular filtration has not been greater than one-tenth of the blood flow, and the amount of fluid reabsorbed has not been less than 75 p.c. of that filtered through the glomerulus.

During an experiment it has been noticed that, while the rate of urine formation may increase, the rate of formation of glomerular filtrate diminishes, but the proportion of it which is reabsorbed is smaller.

It is intended in further experiments to compare the rate of elimination of creatinine with that of some non-metabolized sugars.

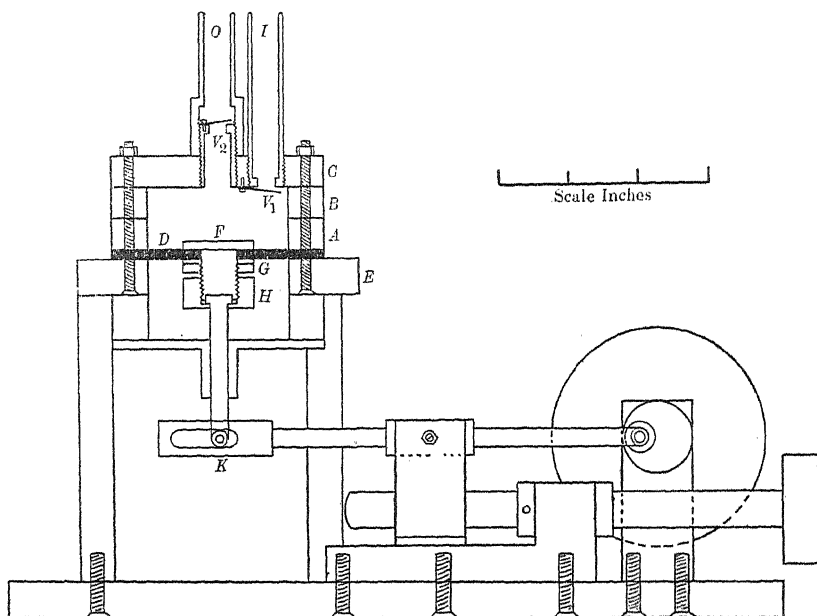
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A new perfusion pump. By A. HEMINGWAY.
(*Physiology Institute, Cardiff.*)

The introduction of erinoid¹ has made possible the construction of a perfusion pump having a solid cylinder which does not expose blood to a metal surface.

The design of the pump, which is of the rubber diaphragm type, is similar in its principles to that introduced by Dale and Schuster [1928], but obviates the use of a glass blood chamber from which the blood is propelled by the movements of a rubber finger stall.



The pump cylinder is built up of two rings (*A* and *B*) of $\frac{1}{2}$ in. erinoid covered by a top plate *C*. The rings are clamped together and held to a base ring by bolts passing through their thickness. The top plate carries the inlet and outlet tubes (*I* and *O*) and their valves (*V*₁ and *V*₂). The tubes and valve seatings are made in erinoid, the valve flaps are cut from thin sheet rubber and secured to the valve seating by 14 B.A. screws. The heads of the screws are covered with celluloid varnish.

The circular diaphragm, *D*, is cut from sheet rubber $\frac{1}{8}$ in. thick and is firmly clamped round its periphery between the erinoid ring *A* and the

¹ Erinoid is a coagulated casein product and may be obtained in sheets or rods from Erinoid Ltd., Stroud, Gloucester.

metal ring *E*. Connection is made to the piston rod through an erinoid plug *F*, which is clamped to the rubber by the screwed ring *G*, and to the driving rod by the flanged fitting *H*.

The method of varying the stroke while running is by alteration of the fulcrum which works in a slide. The thrust from the crank to the piston rod is transmitted through the fork *K* and a roller bearing.

Direct comparison of this pump with a glass-and-rubber pump of the finger-stall type [Hemingway, 1931] was made by changing from one to the other during an isolated kidney perfusion. There was no change in the blood flow through the perfused organ when the change-over was made.

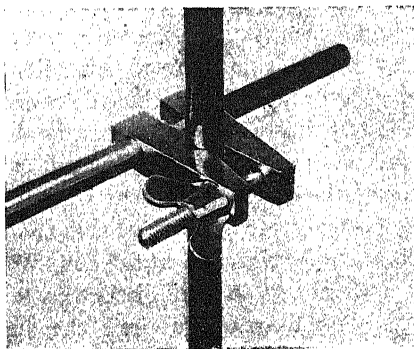
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A one screw-fixing boss head. By A. HEMINGWAY and A. L. SIMS. (*Physiology Institute, Cardiff.*)

The boss head described has the advantage that it can be moved to any position and both rods held in it clamped with great rigidity and at right angles by tightening only one screw.

The method of construction is shown in the diagram. Two similar rectangular blocks of brass, 2 in. \times $\frac{1}{2}$ in. \times $\frac{1}{2}$ in., have a $\frac{3}{8}$ in. diam. hole drilled with centre $\frac{3}{8}$ in. from one end. A V-groove is cut in the middle of the inner face at right angles to the hole. Beyond the groove, holes are drilled to take the clamping screw, and the faces are cut away so that the blocks can be held at an angle to each other when clamping small rods.



Modification of speech sounds in passing through acoustic filters.

(Demonstration.) By F. H. GAGE and J. H. SHANBY.

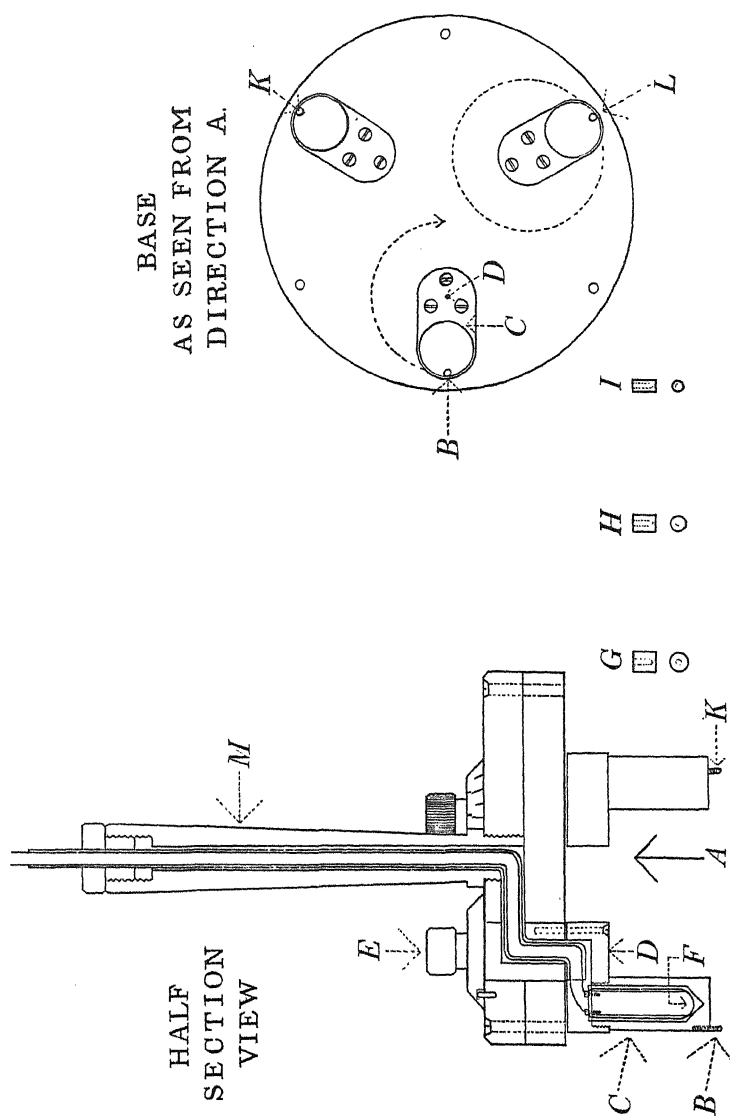
The type of hearing associated with high-note deafness can be roughly imitated by the sounds transmitted by a speaking tube in which is included a low-pass acoustic filter. The high notes associated with consonants and, to a less extent, with most of the vowels are by this means greatly weakened, and the resulting speech sounds, though audible, are so distorted and their characteristic differences so much reduced that they are only with difficulty intelligible.

The acoustic filter used in the demonstration consists of a brass tube forming part of the conduit through which the sounds pass, pierced with a series of rings of holes, each ring opening into a separate short cylindrical chamber, concentric with the central brass tube.

The dimensions of these cylinders are such that notes higher than about 800 ~ are cut off, lower tones being transmitted with very little loss, while those above the cut-off lose twenty or more decibels in passage through the filter.

A new differential æsthesiometer. By T. GRAHAM BROWN.

The object of this instrument is to test the possibility of the sensory localization of a heat stimulus as such. Three similar metal discs (the surface areas of which may be varied in different experiments—discs of diameters between 1.5 mm. and 6.0 mm. are used) are so arranged that they may be placed equi-distantly, and at any selected distance between 6 mm. and 80 mm. apart. One of the three discs contains a resistance wire which is capable of heating it to different temperatures by the passage of an electric current. There is no outward indication on the instrument of the disc which may be so heated. The three discs are placed on the skin of a subject at a selected distance apart—one disc having been heated to a desired temperature. As all other stimuli (such as pressure and touch stimuli) are common to the three discs, if the subject experiences a heat sensation, and can localize it at the area of skin touched by the heated disc, he then localizes a sensation of heat as such. The discs are capable of being arranged not only in an equi-distant pattern, but also in any other which falls within the area of a circle the diameter of which is about 90 mm.



Differential aethesimeter. *B*, rod (provided with screw) forming smallest contact disc and inserted in margin of metal cylinder. *C*, metal cylinder, which is capable of rotation round point *D* by means of milled head *E*. *F*, resistance wire enclosed in cylinder *C*. *G*, *H* and *I*, discs of different diameters capable of being screwed on to *B*, *K* and *L*. *K*, and *L*, contact discs similar to *B*, but not capable of being heated. *M*, handle of instrument containing electric leads to resistance wire in *C*.

The regions of greatest energy in the production of vowel sounds. (Demonstration.) By F. H. GAGE and J. H. SHAXBY.

The sites of disturbance of the vocal cavities and tissues concerned in the production of vowel sounds can be conveniently studied by a telephone ear-piece used as a microphone and applied to the mouth or throat. The telephone is connected to a rectifying valve circuit of the Moullin voltmeter type, so that the energy supplied to the telephone is manifested by the deflection of the galvanometer, and shown by the movement of a light-spot on a scale.

The vowel sounds fall into two classes, those in which the energy is mainly expended in the air vibrations at the more or less opened buccal aperture, and those in which it is associated with powerful vibrations of the vocal cords and neighbouring muscles.

A (as in "father") is the type of the former class, showing strong deflections when the telephone is held just in front of the mouth, but none when it is pressed upon the side of the throat, slightly higher than the thyroid cartilage. *E* (as in "feet"), on the contrary, gives a strong deflection with the telephone in the latter position, but practically none with it in front of the mouth.

The other long vowels show intermediate distributions of the energies manifested in the two positions of the telephone. They lie between the two extremes in the order: *E* (feet), *Ū* (boot), *O* (boat), *AW* (fault), *Ê* (fate), *A* (father), where each gives less oral response and more throat-muscle response than the one which follows.

The vowels may be spoken or sung; change of pitch, though altering the intensity of the response, in no way affects the general relations described.

Degeneration of peripheral nerves after spinal transection in the monkey. By SYBIL COOPER and C. S. SHERRINGTON.

In the monkey (*e.g.* Macaque) total spinal transection in the mid-thoracic region brings frequently as a sequel Wallerian degeneration of nerves in the hind limb (1), (2), (3). In a series of eight experiments of this kind we have found Wallerian degeneration of nerves of the hind limb in five of them. Clean rapid postoperative healing without a trace of sepsis has occurred in every case, and care and attention to the animal have avoided the incidence of bedsores. There has been no complication by myelitis; therefore the possibility of myelitic degeneration of the motor horn cells as accounting for Wallerian changes in the fibres in the limb

nerves is excluded. A feature presented by the peripheral degeneration is that in some cases in certain of the affected nerve trunks not a single myelinated fibre remains sound. In our experience the degeneration is always confined to the sciatic nerve and its branches. A character observed has been the striking asymmetry in the amount and distribution of the degeneration in the nerve trunks of the right and left limbs. Thus the degeneration may be very slight in certain nerves of one limb and entirely destructive of those same nerves in the other limb.

In three only of these experiments have the spinal nerve roots of the hind limb, as well as the limb nerves themselves, been examined for Wallerian degeneration. The search revealed no trace of degeneration in either the ventral or dorsal roots, although the degeneration in nerves of the limb was very heavy. In these three experiments the periods allowed were four, seven and two weeks respectively. Notwithstanding absence of degeneration in the spinal roots, the sciatic nerve trunk was heavily degenerated even as far proximal as the level of the hip joint.

An inference to be drawn is that some traumatic damage is likely to supervene in the sciatic nerve in the monkey in a paraplegic state, although the animal be provided with soft hay bedding in a roomy cage.

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PROCEEDINGS

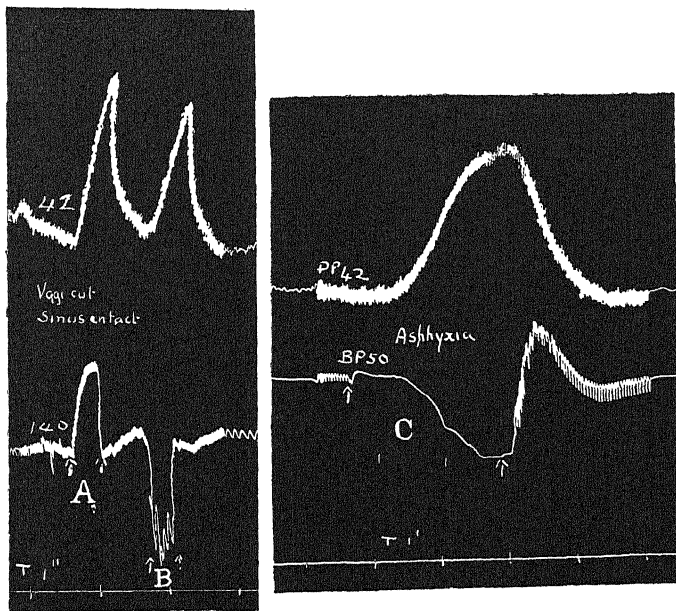
OF THE

PHYSIOLOGICAL SOCIETY,

December 17, 1932.

A convenient method of studying the reactions of the vaso-motor centre. By R. J. S. McDOWALL. (*Dept. of Physiology, King's College, London.*)

The method consists of a combination of that used by Bartlett and that demonstrated by me to the Society in 1923 and subsequently described by Hemingway. It consists essentially of recording the perfusion resistance of a part of the body, the blood vessels of which



have been tied off for the most part from the general circulation. The apparatus can be made to give magnification several times that of the blood-pressure variations, and all the ordinary reactions of the vaso-motor centre may be shown with complete readiness and uncomplicated by the cardiac or peripheral vascular effect. The figures indicate typical

records obtained by the method. The upper tracing in each instance indicates perfusion resistance in the hind limbs of a cat, the lower, blood-pressure in the left carotid artery, the vagi being cut in each case. At *A* the right carotid artery was clamped, *i.e.* the carotid sinus clipped off, temporarily between the arrows, at *B* the heart was held. These records show the reaction of the centre to fall of arterial pressure. In *C* the animal was asphyxiated between the arrows. The stimulation of the vaso-motor centre due to the asphyxia is seen in the perfusion record, although it is completely masked in the record of arterial pressure. That the two circulations are sufficiently separate to warrant the results being ascribed to the vaso-motor centre is shown by the fact that these reactions disappear after denervation or loss of reaction of the vessels of the part. The method for many investigations is an excellent substitute for the plethysmograph.

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The rate of diffusion into erythrocytes. By MONTAGUE MAIZELS.

In re-investigating the permeability of erythrocytes to phosphates [Maizels and Hampson, 1927] it was found necessary to determine the rate at which the anion diffuses. As the rate of phosphate diffusion varies with temperature, the temperature coefficient of phosphate diffusion was first studied. Heparinized blood was centrifuged and the cells mixed with one hundred times their volume of potassium phosphate solution of constant *pH* at various temperatures. After 5 minutes the suspensions were centrifuged in a series of tubes drawn out into sealed graduated capillaries. The tubes were then washed without disturbing the red cells in the capillaries. The volume of red cells was read and their phosphorus content estimated. At *pH* 8.4 and *pH* 5.4 the temperature coefficient between 10° and 20° C. was 1.7; between 20° and 30° C., 2.4; and between 30° and 40° C., 1.15.

Rate of Diffusion. If cells are suspended in mixtures of H_3PO_4 , K_2HPO_4 and KH_2PO_4 , $\Delta = 0.42^\circ \text{C.}$, the maximum phosphorus content after 1 hour at 24° C. is at *pH* 5.3. But if the phosphorus content is measured after only 5 minutes' exposure it will be found that this is maximal at *pH* 5.7. This indicates that phosphate permeates most easily at *pH* 5.7 (Table I).

If KCl ($\Delta = 0.42^\circ \text{C.}$) is added to the phosphate it is found that the permeation of phosphate is greatly depressed; but the entry is most rapid at pH 6.

TABLE I.

Cells in pure phosphate solutions				Cells in mixtures of potassium phosphate and chloride ($\Delta = 0.42^\circ \text{C.}$)	
pH	External P mg./100 cc.	Cell P		External P	Cell P at 60 min.
		5 min.	60 min.		
3.6	402	78	275	—	—
5.1	396	160	340	198	32
5.3	390	180	352	195	35
6.0	367	192	325	183	68
6.2	358	155	290	179	60
6.7	337	105	230	168	45

When 0.05 c.c. of cells is suspended in a mixture of equal parts of KCl, and KH_2PO_4 , the resulting pH is about 5.1, and the cell chloride is 112 millimoles, compared with 62 mm. in the external fluid. It appears that excess of chloride enters the red cell to balance the osmotic pressure of the external chloride and phosphate, and to satisfy the anion requirements of the red cell at pH 5.1. If cells are placed in a mixture of KCl (1 part) and KH_2PO_4 (5 parts), the external chloride is 21 mm. and the internal Cl 102 mm. This preponderance of cell chloride is brought about by an excess of any relatively indiffusible anion or non-electrolyte in the external solution.

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Impulses in the giant nerve fibres of earthworms. By J. C. ECCLES,
R. GRANIT and J. Z. YOUNG.

In the ventral nerve cord of the earthworm there lie three very large non-medullated nerve fibres, and it has recently been shown that in the living worm the middle of these conducts antero-posteriorly and the two lateral fibres postero-anteriorly [Stough, 1926, 1930]. The fibres are not continuous throughout their length, but are divided into segments by membranes which apparently resemble the surface membranes of the fibres. The surfaces of contact are usually not flat, but curved in various ways so that the concave end of one segment fits over the convex end of its neighbour.

Impulses in the nerve cord can be recorded by a Matthews' oscillograph when the head or tail of the earthworm is stroked with a feather.

The conduction of such impulses has been studied in the isolated nerve cord stimulated by induction shocks. Two impulses are set up by a sufficiently strong shock applied to either the head or tail end of the nerve, the conduction rate of the faster impulse varying in different experiments from 17 to 25 metres a second and that of the slower from 7 to 12 metres a second (temp. 10° to 12° C.).

By altering the strength of the stimulus it can be shown that the impulses have an all or nothing relationship to this stimulus, even the finest adjustment of threshold failing to show that either impulse is made up of two separable impulses. Stough has described transverse connections between the lateral fibres, so it would appear that the presence of only two impulses is due to these two fibres forming a single conducting unit. By stimulating the nerve trunk first at one end and then at the other end a few sigma later and leading from the centre of the nerve to the oscillograph, it has been shown that the fast impulse in one direction travels in the same fibre as the fast impulse in the other direction, and similarly with the slow impulse. Thus, in spite of the transverse membrane, the impulses can pass in either direction. The unidirectional conduction found by Stough is not, as he suggested, due to the transverse membranes; it must be due to the connections of the nerve fibres.

At the head end the threshold stimulus for setting up the fast impulse is much lower than that for the slow impulse, while at the tail end the difference is always less. Also the action potential of the fast impulse at the head end is much greater than that of the slow impulse, but at the tail end the potential of the latter may even exceed that of the former. Since at this end the diameters of the lateral fibres are relatively to the median fibre, greater than anteriorly it seems certain that the slow impulse travels in the lateral fibres and the fast impulse in the median fibre. Experiments on the nerve impulse indicate that the rate of conduction is approximately proportional to the diameter of the fibre, and the action potential to the cross-sectional area. In our experiments the relative velocities of conduction of the fast and slow impulses are in accordance with the relative diameters of these fibres, but the action potential of the slow impulse is relatively about twice the size that would be expected from a single lateral fibre. This doubtless is due to the summed potential resulting from the two lateral fibres conducting as one.

The refractory period and recovery curve have been determined in three experiments, and are approximately the same for the median and lateral fibres. An impulse is conducted more slowly in the relatively refractory period and it has a lower potential. In fact the impulses in the

earthworm nerve have been very similar to the A group of impulses in frog nerve. In such respects, therefore, the transverse membranes have no influence on the conduction of impulses, although the separation of the segments by means of these transverse membranes appears to be as complete as that existing at vertebrate synaptic junctions. However, investigation into the polarization of these nerves by galvanic currents, though still in its preliminary stages, seems to bring out certain differences from vertebrate nerve which may be ascribable to the polarization of these membranes.

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The action of veratrine on frog's nerve. By H. FROMHERZ
and A. V. HILL.

In muscle the prolonged veratrine contraction is associated [Hartree and Hill, 1922] with a prolonged liberation of heat, the ratio (at any moment) of tension to rate of heat production being the same as in an ordinary tetanus.

Nerves soaked in 1/50,000 veratrine and subjected to a short tetanus show a somewhat prolonged heat production: they also give a slight after-potential. In order, however, to obtain the characteristic veratrine effect we have first poisoned our nerves, then asphyxiated them in pure N_2 or H_2 , then allowed them to recover in O_2 . Graham and Gasser [1931] found this treatment to yield a prolonged after-potential. We have observed veratrine after-potentials lasting for minutes following a single shock to a nerve fully recovered from asphyxia. Presumably the drug is unable to penetrate and produce its characteristic effect until the nerve surface is somehow disorganized by oxygen want.

In a nerve veratrinized in this way the heat produced in response to a single condenser discharge may be 100 to 1000 times as great as in a normal nerve. In a succession of shocks at regular intervals the heat per shock is less, depending on the interval, but still far more than normally. The "veratrine ratio" is greatest after prolonged rest and grows less as the period since previous activity diminishes. The second of two shocks, e.g. at 5 sec. interval, may give half as much heat as the first, at 10 sec. interval two-thirds, at 30 sec. interval three-quarters.

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The effect on the body temperature of animals of injections of an extract of parathyroid glands. By M. H. B. ROBINSON and J. H. THOMPSON. (*Research Laboratories of the University of London, King's College and the Royal College of Surgeons, London.*)

It has been shown that extirpation of the parathyroid glands in rats and rabbits results in a disturbance of the heat-regulating mechanism; the injection of parathyroid preparations somewhat compensates for this loss of regulatory function [Ogawa, 1925].

A series of readings has been taken in rabbits and rats which have been injected with an extract of parathyroid glands, free from the factor which raises the blood calcium [Collip]. The preparation of the extract is described by Robinson and Thompson. The temperature readings were taken under standard conditions with regard to room temperature (65° F. thermostat controlled), diet, and time relation to injection. The thermometer was inserted per rectum sufficiently far to cover a fixed mark on the bulb. Daily observations were made. The results are tabulated as follows:

Range of temperature: average of 12 rabbits.					
<i>Rabbits.</i>					
		Weeks	1st	3rd	5th
Experimental	Average maximum		100	100.1	100.2
	Average minimum		99	99.2	99.3
Controls	Average maximum		101.5	101.2	102
	Average minimum		101	100.4	100.8

The average range of temperature during the experimental period was:

Experimental rabbits 99.1 to 100.1

Control rabbits 100.7 to 101.5

The experimental rabbits maintained their body temperature at a lower level than that of the control rabbits throughout the experiment.

Range of temperature.							
<i>Rats.</i>							
		Weeks	1st	2nd	3rd	4th	5th
Experimental	Average maximum		97.3	97.5	98	98.6	98.6
	Average minimum		97.2	97.1	97.2	98.0	98.0
Controls	Average maximum		98.2	98.9	98.6	98.9	98.7
	Average minimum		98.0	98.0	98.1	98.3	98.4

The average range of temperature during the period in which injections were made was:

Experimental rats 97.2 to 97.6

Control rats 98.0 to 98.6

Injections were discontinued after the end of the third week, and the

temperature of the experimental animals began to rise three days after cessation of injection. From that time up till the last readings the temperature of the experimental rats has closely approximated that of the control animals.

A preliminary experiment indicates that injection of extract of anterior lobe of pituitary gland can raise the temperature of animals in which it has been lowered by injection of a parathyroid extract as described.

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The effect of veratrine on the electrical response of crab's nerve.

By S. L. COWAN. (*Marine Biological Laboratory, Plymouth.*)

With a Cambridge Instrument Company's A and M galvanometer (sensitivity 1 mm. = $4 \cdot 10^{-10}$ amp. at 3 metres, and period 3.8 sec.) a spider crab's (*Maia squinado*) leg nerve gives an action current deflection of about 30 mm. in response to a single just maximal shock [A. V. Hill, 1932, and Beresina and Feng, 1932], provided by a condenser discharge.

Usually, after dissection, the nerve is allowed to rest for an hour in aerated sea water to avoid the onset of reversible inexcitability; in the present experiments it was transferred at the end of the first half hour to sea water to which had been added previously the required amount of veratrine hydrochloride solution, any carbon dioxide liberated being blown off by passing air through. With such dilute veratrine solutions as were used, 1 in 10 millions to 1 in 100 millions, the pH of the sea water need never be altered by more than 0.1 unit, and control experiments showed that nerves soaked in sea water, which had been acidified with hydrochloric acid and the carbon dioxide removed by blowing in air, gave responses which did not differ from those given by nerves soaked in untreated sea water. At the end of the second half hour the nerve was mounted, the peripheral end crushed and galvanometer connections were made *via* non-polarizable electrodes. After oxygen had been passed through the chamber for fifteen minutes to allow the nerve to recover from the crushing, readings were taken to get a base line and then the nerve was stimulated at the central end. The initial action current response of a nerve so treated with veratrine was very large, as much as

650 mm., and the area of the deflection time curve was increased more than a hundredfold. The "retention" of action current described by Levin [1927] and by Furusawa [1929] lasted for nearly half an hour.

The optimum concentration of veratrine, for autumn crabs, lies in the region of 1 in 25 millions to 1 in 50 millions. A definite fall in the response can be noticed with concentrations of 1 in 10 millions on the one side or 1 in 100 millions on the other; in the latter case the response of these non-medullated nerves is still about fifty times larger than normal. Asphyxiation after soaking in veratrine sea water and previous to stimulation in oxygen does not affect the response.

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Loss of heat at high altitudes. By BRYAN H. C. MATTHEWS. (*Physiological Laboratory, Cambridge.*)

At sea level, in dry cold air, 15–25 p.c. of the total heat production of the human body is dissipated through the lungs and respiratory passages, in vaporizing water, setting CO₂ free from solution, and warming air. At high altitudes a much higher percentage of the total heat produced must inevitably be lost from the lungs, etc., for the heat loss per litre of respired air remains about the same while the potential heat gain in the form of oxygen becomes less and less. The rapid respiration necessary to obtain adequate oxygen may dissipate heat as fast as it can be gained by utilizing that oxygen.

If these effects are considered quantitatively some surprising results come to light. The heat loss per litre of air at -10° C. respired (whether dry or saturated makes little difference) is approximately 27.5 cal. in water vapour, 2.5 cal. in CO₂ and warming air. *Thus total heat lost per litre of air respired is 30 cal.* From 1 c.c. of oxygen absorbed about 5 cal. can be produced. For each millimetre by which the tensions of oxygen in inspired and expired air differ, approximately 1.3 c.c. of oxygen are absorbed from each litre respired, and 6.5 cal. are available. *Total heat gained per litre respired and per mm. difference in oxygen tension in inspired and expired air, 6.5 cal.*

As the barometric pressure falls the oxygen tension in inspired air falls, that in alveolar air also falls, that of the expired air will always be a little above that of the alveolar sample owing to dead space, etc. With fall of barometric pressure the difference between the oxygen tensions in inspired and expired air becomes smaller and smaller.

It will be seen from the above figures that unless the inspired and expired air differ in oxygen tension by about 5 mm., even if clothing is so perfect that it prevents any heat loss from the skin, the body temperature cannot be maintained. By extrapolation from figures for alveolar air up to 23,000 ft. [Barcroft, 1925; Somervell, 1925] it appears that these conditions will be realized somewhere round about 30,000 ft. Human life above the point where this occurs will be impossible without respiratory aids or artificial heating, for the body temperature must fall.

I have constructed several forms of respirator to recover the heat and water vapour from air expired and return it to the inspired air; these are at present undergoing tests. It is hoped it may be possible to prevent 40-80 p.c. of the total heat loss at high altitudes, and to eliminate the throat irritation resulting from desiccation.

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Decreased urinary flow in rats on high fat diet. By CASIMIR FUNK and P. P. ZEFIROW. (*Casa Biochemica, Rueil-Malmaison, France.*)

In connection with the work on the hormones appearing in urine [Casimir Funk and P. P. Zefirow, 1932] an interesting observation was made, which apparently so far has escaped the attention of investigators and clinicians. A series of rats were kept on a practically fat-free diet on one hand, and on a high-fat diet on the other. Without any noticeable difference in the water consumption in the two series of animals, and with the food consumption lowered only by 40 p.c. in average, the urine excretion was much lower in the high-fat series. In a few instances an addition of 125 g. of butter to the usual (but not controlled) diet in human beings caused a diminution of urine volume by about 20 p.c. No explanation can be offered at present, but it seems possible that a high-fat diet may influence the pituitary gland.

The animals used were rats, two males and one female kept together

in a metabolism cage. Altogether 48 young growing animals were used. Water and food were given *ad libitum*. The diets used consisted of 24 g. caseinogen, 62 g. starch, 10 g. dried yeast, 4 g. salt mixture, and 2 c.c. of cod-liver oil. In the high-fat diet 48 g. of starch were replaced by butter. In the last recorded experiment the fat content was increased to 73 p.c. and lard used instead of butter, without change in the results obtained. The first two experiments were performed during the heat of the summer, the last two in autumn and winter. In summer the urine volume was lower, due to increased loss through the skin. The results are summarized in the table below:

Exp.	Duration of experiment, days	Fat-poor diet: daily variations c.c.	Average c.c.	High-fat diet: daily variations c.c.	Average c.c.
I	8	22-30	26.2	1-6	3.3
II	14	20-53	32	1-11	3.7
III	10	14-34	26.1	11-24	14.2
IV	41	12-44	28.7	1.5-11	6.0

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Action of ether on the sympathetic system. By B. B. BHATIA and J. H. BURN.

Elliott [1912] showed that ether, chloroform and urethane caused a discharge of adrenaline from the suprarenals, which occurred so long as the splanchnic nerves were intact. We have investigated the action of ether on other parts of the sympathetic system, making our observations on cats from which the suprarenals were removed and using the decerebrate preparation with vagi cut, and also the spinal preparation.

When ether vapour is mixed with the air used for artificial respiration, there is a powerful contraction of the spleen, inhibition of intestinal movements and tone, inhibition of uterine movements and rise of heart rate. These effects are reversed as soon as the ether is discontinued. In animals in which the spinal cord is destroyed, these effects are not seen, except that a gradual paralysis of intestinal movements occurs which persists long after removal of the ether. The contraction of the spleen in the decerebrate cat is abolished when the sympathetic ganglia are paralysed by nicotine.

As shown by heart-lung experiments, ether weakens the heart more than is commonly supposed, but this is not obvious in the intact animal

because of a simultaneous vaso-constriction, presumably due to sympathetic impulses. Thus an amount of ether which has no effect on the blood-pressure of a decerebrate cat causes a profound fall of blood-pressure after paralysis of the sympathetic ganglia by nicotine. Since ether has no appreciable action on the blood vessels themselves, as shown by perfusion experiments, this fall represents the uncomplicated effect on the heart. It is concluded that ether causes a discharge of impulses along all sympathetic nerves.

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Electric symmetry of the thorax. By W. A. OSBORNE (*Melbourne*).

When the two hands are led off to the string galvanometer, as in the conventional lead I, and when the string is adjusted to give the sensitivity required for standard electrocardiograms, it will be found that normally a deep breath produces either a very small deflection or none at all. As is well known a slight movement of either arm or hand develops a current of several millivolts. Lack of symmetrical expansion of the thorax in deep breathing can be readily detected in this way, and it is rather curious that, though the diaphragm is not bilaterally symmetrical in the normal human being, no potential difference of any magnitude is produced. A remarkable fact is that all the left-handed students I have examined, both male and female, show on taking a deep breath a string deflection of several millivolts amplitude pointing to some disturbance of the normal balance of effort in the right and left halves of the thorax.

PROCEEDINGS
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January 21, 1933.

The extraction of histamine from tissues by electrodialysis.
By R. G. MACGREGOR and W. V. THORPE. (*Preliminary communication.*)

A simple method for the estimation of very small amounts of histamine in tissues would be of great value in studying the rôle of histamine in the body. Existing methods involve a laborious chemical extraction of the histamine from the tissue followed by a physiological assay of the extract. (Colorimetric estimation of histamine in tissue extracts by the Pauly reaction cannot be accepted as reliable.) The most convenient and reliable physiological method for the assay of histamine is matching against a standard solution of pure histamine on the blood-pressure of an anaesthetized cat.

Whilst our main aim, the substitution of a reliable chemical method for the physiological one, has not been achieved, we have effected a very considerable saving of time in the preliminary chemical manipulation. The alcohol method [Best, Dale, Dudley and Thorpe, 1927] requires attention during three days; the more drastic HCl method [Best and McHenry, 1930] requires one day. By our method of electrodialysis in a three-compartment cell histamine can be obtained from a tissue ready for injection within an hour and a half. The solution is clear and colourless whereas by the other methods it is coloured and often cloudy. No reagents apart from distilled water and a little acid for the final neutralization of the cathode liquid are necessary. The temperature can be kept below 35° C.

So far our experiments have only been concerned with blood and lung. In the former there is no histamine, but histamine added in varying amounts has been recovered practically quantitatively. In one experiment the histamine was easily isolated as picrate from the dialysate by drying with sodium carbonate and extracting with chloroform. In a number of experiments with lung (ox and dog) portions of the same mince were extracted by electrodialysis and by the alcohol method. In every case the dialysates and the control alcohol extracts had identical effects on the cat's blood-pressure. Although the histamine cannot be estimated by the Pauly reaction, the passage of histamine into the cathode chamber

has been followed by a modification of the reaction, in which *p*-nitraniline is used in place of sulphanilic acid. When all the histamine has been extracted the intensity of this Pauly reaction (measured in a Rosenheim-Schuster colorimeter) remains constant. The reliability of this indicator has been confirmed by assays on the cat's blood-pressure during some of the dialyses. The experiments, in addition to supporting the view that histamine in the tissues is either free or very loosely combined, confirm the reliability of the alcohol method used in previous experiments [Best, Dale, Dudley and Thorpe, 1927; Thorpe, 1928, 1930].

The apparatus is in essentials similar to that of Foster and Schmidt [1923], the cells being made of waxed wood to take 20 or 50 g. of tissue. The membranes are ordinary parchment dialysing paper and the cathodes of pure nickel sheet.

The experiments are being extended to other tissues and it is hoped to publish full experimental details on their completion.

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“Vaso-tonins” and the pump-oxygenator-kidney preparation.

By L. E. BAYLISS and E. OGDEN.

A. In order to discover the relative toxicity of heparinized and defibrinated blood, and the effect of time on the development of vaso-tonins, small quantities (about 10 c.c.) of the different bloods under test were added to a pump-lung-kidney preparation (containing 150–200 c.c. blood) and the rise in perfusion pressure recorded. The following conclusions were reached:

(1) Heparinized blood is not significantly different from defibrinated blood which has been properly filtered (through six thicknesses of butter muslin).

(2) The vaso-tonins develop immediately, *i.e.* within 2 min. of removing the blood from the animal.

(3) There is no significant increase in vaso-tonic activity with time up to $1\frac{1}{2}$ to 2 hours, and it is immaterial whether the blood be kept warm or allowed to cool to room temperature.

(4) Up to $1\frac{1}{2}$ to 2 hours after being shed, the rise of pressure produced passes off completely in 10 min. Blood kept for a longer time than this

produces a permanent rise in pressure which, as far as we can see, never completely passes off. These vaso-tonins, apparently, cannot be removed by a pump-lung-kidney preparation.

(5) Neither "temporary" nor "permanent" vaso-tonic activity is affected in any way by filtering the blood through fine cambric (50-70 μ pores).

(6) Defibrinated blood kept for 48 to 120 hours loses the "permanent" vaso-tonins, but retains the "temporary."

(7) Defibrinated blood which has been circulated through a pump-lung-kidney preparation regains its "temporary" vaso-tonins on being allowed to stand for an hour. It never develops "permanent" vaso-tonins.

(8) In the presence of a sufficient quantity of ergotoxin to inhibit the action of adrenaline (10 γ per 100 c.c. blood), almost any quantity of even the most toxic preparations can be added without resulting in any vaso-constriction (confirming the observations of Heymans, Bouckaert and Moraes [1932]).

B. If a kidney is to be perfused satisfactorily without simultaneous perfusion of lungs or other organs, the blood must be detoxicated by the kidney itself. We have accordingly constructed a perfusing circuit which, including the oxygenator, requires only 70 c.c. blood for an average-sized dog's kidney. The oxygenator consists of a piece of rubber tube about 1 metre long and 5 mm. inside diameter, connected between the outlet from the kidney and the blood reservoir. The reservoir is closed and connected to a vacuum pump, the vacuum being so adjusted that alternate drops of blood and bubbles of air (or oxygen) pass down the rubber tube. The uptake of oxygen is remarkably rapid, and the froth formed consists of large bubbles, and so breaks easily.

Kidneys perfused with this apparatus, however, never reach more than a half or three-quarters of the blood flow of these perfused in a pump-lung-kidney preparation, so that it seems probable that detoxication is never quite complete. The inclusion, from the beginning, of a fine cambric filter in the circuit did not result in any larger blood flow (in contradistinction to the observations of Daly and Thorpe [1932]). The urine flow, however, is usually quite good, and quite high concentration ratios may be obtained (exp. 5, on heparinized blood gave a maximum urine flow of 0.76 c.c. per min., and a creatinine concentration ratio of 30, the urine containing 0.09 *M* chlorides).

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A seven horse-power Austin engine adapted as a blood pump.

By I. DE BURGH DALY.

The perfusion of the whole or portions of one or more large animals (dogs) in cross-circulation experiments necessitates larger perfusion pumps than those at present in general use. Two Dale and Schuster pumps in parallel are able to put out sufficient blood for perfusion of the whole systemic circulation of a small dog, but they are working to the limit of their capacity when it is desired to raise the systemic arterial pressure to a value higher than normal.

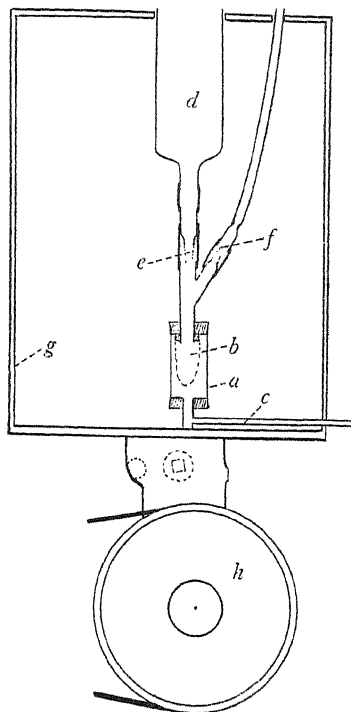
The apparatus described consists of four separate pumps, each of which is capable of dealing with considerable blood flows, and it has been found to be satisfactory for cross circulating large animals. A table is given below of the outputs at different pressures and frequencies.

Rate per min.	B.P. mm. Hg.	Maximum output of each pump, litres per min.
100	50	4.0
100	175	3.1
100	220	1.8
150	120	5.1
150	220	2.7

It will be seen that the output diminishes as the pressure rises, due to compression of the air in the transmission system, but except under certain circumstances this is not a disadvantage since the output of each pump can be adjusted whilst running. The figure shows one of the pump units mounted above the engine.

The inlet and exhaust port holes of the engine are closed and the four cylinders used as compressors by driving with an electric motor. The cylinders are in communication with glass chambers (*a*), containing heavy latex finger stalls (*b*) supplied by Macinlop, Manchester. The finger stalls are alternately compressed and decompressed by the rise and fall of the pistons, air transmission being used. The degree of compression and consequently the output of the pump are controlled by adjusting the opening of a side tube (*c*) communicating with the cylinders. The glass reservoir (*d*), inlet and outlet valves (*e*, *f*) are so arranged that no air is trapped in the pump circulation when blood is poured into the reservoir. The blood does not come into contact with any metal parts. The unit (*b*, *d*, *e*, *f*) is fixed by rubber-covered clamps (not shown in the figure) designed for quick removal for the purpose of cleaning. A glass-walled thermostat (*g*) kept at 37–39° C. surrounds the glass part of the pumps and is of sufficient size to prevent excessive local heating by the electric

lamps. The specification of the electric motor supplied by the Hockley Chemical Co. Ltd., is as follows: volts 220; amps. 2.5; H.P. 0.5; R.P.M. 500. A series resistance controls the frequency of the motor. A $2\frac{1}{4}$ in. diameter flat pulley wheel drives on to the $9\frac{5}{8}$ in. diameter engine fly-wheel (*h*) by means of a 1 in. wide flat belt. The glass connection tubes are $\frac{1}{2}$ to $\frac{3}{4}$ in. internal diameter and the valves $\frac{5}{8}$ in. flat rubber tubing.



Survival after adrenalectomy in rabbits. By CHARLES REID.

In a series of thirty-two rabbits, mainly of the albino Himalayan variety, adrenalectomized through the lumbar extraperitoneal route by aseptic operations at intervals of not less than 16 days, four died within 2 or 3 days, nine died between the 9th and 17th days with signs of adrenal insufficiency, and nineteen survived for long periods (over 100 days) still in apparently good health. This indicated that, in a series of successful double adrenalectomies, 60 to 70 p.c. of rabbits of the above species

may be expected to survive for an indefinite period. In this connection Kojima [1929] found accessory cortical tissue in 70 p.c. of normal rabbits. The above result is much better than that reported by Dominguez and Rogoff [1924] in which only three rabbits out of nineteen survived more than 30 days.

The adrenalectomized rabbits which died between the 9th and 17th days seemed normal on inspection until 2 or 3 days before death when loss of appetite, weakness and lassitude became evident. Before the onset of extreme terminal symptoms, other signs of adrenal insufficiency were (1) reduced fasting blood sugar p.c. as compared with normal; (2) hæmoconcentration; (3) loss of body weight (average 20-25 p.c. at death); (4) slow and feeble heart beat.

A fall in body weight was the most convenient and reliable guide to the probability of signs of adrenal insufficiency supervening.

TABLE I. Blood sugar p.c. Specific gravity.

Before adrenalectomy	0.102	1.048	Average for nine rabbits dying between 9th and 17th days after second operation.
After "	0.078	1.061	
Before "	0.106	1.050	Average for nineteen long-survival rabbits.
After "	0.098	1.052	

The series of thirty-two rabbits comprised thirteen males and nineteen females. Sex and weight did not seem to be a determining factor in the survival of double adrenalectomized rabbits (Table II).

TABLE II.

	No. of rabbits	Male	Female	Average wt. in kg.	
	32	13	19	1.45	1.50
Early deaths	4	2	2	1.42	1.49
Deaths from acute adrenal insufficiency	9	3	6	1.46	1.49
Survivals	19	8	11	1.45	1.51

In view of the frequent occurrence of œstrus in the rabbit and the striking prolongation of life after adrenalectomies reported in dogs in heat by Rogoff and Stewart [1928] in a small series of cases, this was not the result expected. It would be difficult in rabbits to attempt to establish the apparent relationship suggested by some writers between ovaries and adrenals in face of the already high percentage of survivals obtained in successful total adrenalectomies in both sexes.

Normal pregnancy and parturition with living young occurred in two adrenalectomized rabbits which were tested from the above series.

In a few rabbits, adrenalectomized apparently when immature, growth and increase of weight were observed. One male, for example,

increased in weight from 1.22 to 1.52 kg. in the course of 100 days after the second operation.

Signs, probably of chronic adrenal insufficiency, were seen after several weeks in four out of thirty rabbits in which one adrenal was removed and the other dealt with by denervation or medulliadrenalectomy for other investigations summarized previously [Reid, 1932]. They were some loss of weight and of hair (four), thickening and desquamation of skin (face and eyelids particularly) (three), flaccid paralysis of hind limbs (two).

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The future of the cathode-ray tube in physiology.

By H. HARTRIDGE.

The cathode-ray tube could be employed in several present-day problems if records could be obtained at somewhat higher frequencies than those obtainable at present. Among these problems may be mentioned the calibration of fast chronaxie meters, the solution of the Wever-Bray problem, the accurate recording of speech sounds, noises, etc., and the accurate recording of impulses in composite nerve trunks. Possible directions of improvement are: brighter cathode-ray tubes, lenses of larger aperture, more sensitive films and greater rates of film travel.

With regard to the first, a brighter spot can be obtained on the end of the tube by using a higher gun voltage, but this causes in time disintegration of the fluorescent material. Two practical points may be made: (1) only to apply full voltage during the actual exposure, (2) to cause the cathode ray to travel successively over different parts of the fluorescent surface. This may be done by causing the cathode ray, which is oscillating horizontally, to move slowly vertically down the fluorescent surface. It may be practicable in the future to cause a film coated with the fluorescent material to move past the cathode ray inside the tube.

The lens system. I have pointed out elsewhere that it is advantageous for this to be of large aperture, and for it to project a reduced image of the end of the cathode tube into the camera. Cinema lenses, as at present

made, have not such large apertures as microscope objectives and condensers. There is a definite need for a special lens to be developed for cathode-ray work. Probably the best lens at present available is an achromatic substage condenser of 0.8 N.A., and about 1 in. focal length. Auxiliary lenses will be needed to flatten the field, and to reduce spherical aberration to a minimum. As made at present, the cathode-ray tube presents a convex surface towards the lens. It would be much better for this surface to be concave. This might be achieved by coating a piece of glass of suitable contour with the fluorescent material, and for this to be mounted inside the cathode-ray tube.

The camera. Super-speed cinema film is available in 16 mm. size. It would be difficult to pass this through the gate of an ordinary cinema camera at speeds of between 10 and 100 metres per sec. An alternative method is to wrap the film round a rapidly rotating drum. A drum of 16 cm. driven at a speed of 20 to 200 revolutions per sec. would provide the required speed. The record obtained would be $\frac{1}{2}$ metre long, and would be photographed in $\frac{1}{20}$ sec. If longer records are required they could be obtained by winding the film spirally round the drum, and arranging for the lenses to move along sideways, so that the image should fall on the centre of the spiral as the drum rotates. Ten spirals would provide a 5 metre record of $\frac{1}{2}$ sec. duration. A possible future development is to cause the cathode ray to fall directly on suitable sensitized photographic material, the whole camera being exhausted prior to the exposure.

High-speed chronaximetry. Since spring-driven chronaximeters may have arm speeds of about 10 metres per sec., film speeds of 10 to 100 metres per sec. are required for the calibration of such instruments. It may be possible in the future to make the cathode-ray tube itself act as a chronaximeter. This may be explained as follows: the cathode "ray" consists of negatively charged electrons, and when these fall on a metal surface, a current flows to this surface from earthed parts of the electrical circuit. Consequently, if one electrode be connected to this plate, and the other to earth, a current flows during the time that the cathode ray falls on the plate. The strength of the current depends on the number of the electrons received in unit time (the "intensity" of the beam), the duration of the current depends on the size of the plate and rapidity of the swing of the cathode-ray. It is known that very rapid movements are possible and therefore very high speeds should be available.

General laboratory purposes. As at present made cathode-ray tubes are large in size, their filaments require a heavy current, and high voltages on

"the gun" are required to successfully work them. Future improvements are likely to reduce these and so make them available for the general measurement of voltages and currents. Since they have no moving parts they would have great advantage over existing types of instruments.

The avoidance of ketosis in the cleidoic egg.

By JOSEPH NEEDHAM.

The excretion of aceto-acetic acid and β -hydroxy-butyric acid is associated, as has long been known, with starvation, severe diabetes, and the restriction of carbohydrate intake. Of these three conditions, the third is eminently observable in the diet of the chick embryo, especially during the last half of its incubation period. On the 17th day of development, for example, the embryo absorbs 16 mg. of carbohydrate, 396 mg. of protein, and 528 mg. of fat (data in Needham [1931 a]). Such a diet should be highly ketogenic, and in fact the "ketogenic balance," calculated according to the method of Hubbard and Wright [1922], is in the neighbourhood of 30 during the last week of incubation. In man the threshold of ketonuria seems to occur at a ratio of 85.

In spite of this very unbalanced diet, however, the chick embryo develops no ketosis. A systematic examination of the allantoic liquid at all stages of development after the 10th day by Rothera's test never gave any indication of the presence of aceto-acetic acid, although the reaction is very sensitive. As ammoniacal nitroprusside does not give evidence of β -hydroxy-butyric acid, and as this acid is often quantitatively the most important of the keto-bodies, further experiments were made in which the van Slyke method [1917] (oxidation to acetone with bichromate and formation of the insoluble mercuric sulphate compound) was applied to allantoic liquid. Here also the results were negative, although small quantities of keto-acid added to the allantoic liquid could be quantitatively recovered.

It might be argued that ketosis depends not so much upon what is absorbed as upon what is combusted. This is not so easy to ascertain, but the embryo, which in the last week of development is 80 p.c. of the living matter in the egg, has certainly then an R.Q. of 0.7, the allantois an R.Q. of 0.8, and the yolk-sac an R.Q. of 0.65 or less. The predominance of fat combustion in this period can hardly be doubted. If, then, it be admitted that the chick avoids ketosis under conditions amply sufficient to provoke it in the mammal, the mechanism of this avoidance becomes a question of great interest. It may be mentioned that a search of the literature has

failed to discover any case of ketosis in an adult bird, even in diabetes. It is possible that immunity to dietary ketosis may have to be added to the list of special adaptations characteristic of "closed box," or cleidoic, development—suppression of protein catabolism, intensification of fat catabolism, uricotelic nitrogen-excretion, and relative shortening of incubation time [Needham, 1931 *b*]. Work on the problem is in progress.

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CONTENTS OF VOL. 78.

No. 1. April 13, 1933.

	PAGE
Nerve endings in mammalian muscle. By BRYAN H. C. MATTHEWS	1
Studies on histamine hypotension. By F. DOMENECH-ALSINA	54
The influence of foodstuffs on the rate of urinary acid excretion. By CHARLES E. BRUNTON	65
The effect of hysterectomy on the oestrous cycle of the ferret. By RUTH DEANESLY and A. S. PARKES	80
The action of strychnine on Hering-Breuer reflexes. By R. S. CREED and D. H. HERTZ	85
Ergotamine and the effect of adrenaline on blood lactate. By M. W. GOLDBLATT	96
The effect of activity on the form of the muscle twitch. By J. L. PARKINSON	106

No. 2. May 23, 1933.

The rate of water absorption in man and the relationship of the water load in tissues to diuresis. By F. H. SMIRK	113
The effect of water drinking on the blood composition of human subjects in relation to diuresis. By F. H. SMIRK	127
The influence of posterior pituitary hormone on the absorption and distribution of water in man. By F. H. SMIRK	147
Note on the subcutaneous absorption of oils by rats and mice, with special reference to the assay of oestrin. By RUTH DEANESLY and A. S. PARKES	155
The effect of sympathetic nerve stimulation on the power of contraction of skeletal muscle. By A. B. CORKILL and O. W. TIEGS	161
Observations on the pumping action of the heart. By HENRY BARCROFT	186
The decerebrate rat. By D. J. BELL, E. A. HORNE and H. E. MAGEE	196
The responses to stimulation of the caudal end of the large bowel in the cat. By R. C. GARRY	208

No. 3. June 12, 1933.

	PAGE
Action potentials in sympathetic nerves, elicited by stimulation of frog's viscera. By SARAH S. TOWER	225
The efficiency of isolated muscle in relation to the degree of aerobic activity. By McKEEN CATTELL and EINAR LUNDSGAARD	246
The action of ether on the sympathetic system. By B. B. BHATIA and J. H. BURN	257
Rhythmic activity in skeletal muscle fibres. By E. D. ADRIAN and S. GELFAN	271
Glycogen synthesis in the small intestine. By E. A. HORNE and H. E. MAGEE	288
The re-synthesis of creatinephosphoric acid in frog's muscle poisoned with iodoacetic acid. By COLIN ASHLEY MAWSON	295
On the supposed inhibitory action of the auricles on the amplitude of the ventricular contractions in the heart of the frog. By ALISON S. DALE	302
The reactivity and activity of the rabbit's uterus during pregnancy, parturition and the puerperium. By J. M. ROBSON	309
Theories of muscular contraction. By A. D. RITCHIE	322
The polymorphonuclear-lymphocyte ratio at an altitude of 5750 feet. By ARTHUR DIGHTON STAMMERS	335

No. 4. July 10, 1933.

The apparent viscosity of blood flowing in the isolated hindlimb of the dog, and its variation with corpuscular concentration. By S. R. F. WHITTAKER and F. R. WINTON	339
The adrenals and anæsthetic hyperglycæmia. By HIRENDRANATH BANERJI and CHARLES REID	370
Alpha and gamma curves in slow muscles. By LOUIS LAPICQUE	381
The influence of ammonium chloride on adaptation to low barometric pressures. By C. G. DOUGLAS, C. R. GREENE and F. G. KERGIN	404
The effects of cholesterol and choline on deposition of liver fat. By C. H. BEST and JESSIE H. RIDOUT	415

CONTENTS.

v

	PAGE
Observations on the retinal action potential with especial reference to the response to intermittent stimulation. By R. S. CREED and RAGNAR GRANIT	419
Size changes in the seminal vesicles of the mouse during development and after castration. By RUTH DEANESLY and A. S. PARKES	442
The effect of the intravenous administration of water upon the rate of urine formation. By W. H. NEWTON and F. H. SMIRK .	451
On the presence of "novadrenine" in suprarenal extracts. By U. S. v. EULER	462
An apparatus for the production of finely dispersed emulsions, and the rate of digestion of fat by lipase in relation to the surface area. By A. C. FRAZER and V. G. WALSH	467

	PAGE
ADRIAN, E. D. and GELFAN, S. Rhythmic discharges in muscle fibres	271
BANERJI, H. and REID, C. Adrenals and anæsthetic hyperglycæmia	370
BARCROFT, H. Pumping action of the heart	186
BELL, D. J., HORNE, E. A. and MAGEE, H. E. The decerebrate rat	196
BEST, C. H. and RIDOUT, J. H. Choline and liver fat with cholesterol	415
BHATIA, B. B. and BURN, J. H. Action of ether on sympathetic system	257
BRUNTON, C. E. Urinary acid excretion after food	65
BURN, J. H. and BHATIA, B. B. Action of ether on sympathetic system	257
CATTELL, McK. and LUNDGAARD, E. Isolated muscle and aerobic activity	246
CORKILL, A. B. and TIEGS, O. W. Sympathetic nerve and skeletal muscle	161
CREED, R. S. and GRANIT, R. Retinal action potential	419
CREED, R. S. and HERTZ, D. H. Strychnine and respiratory reflexes	85
DALE, A. S. Influence of auricles on ventricle	302
DEANESLY, R. and PARKES, A. S. Hysterectomy and œstrous cycle	80
DEANESLY, R. and PARKES, A. S. Subcutaneous absorption of oils	155
DEANESLY, R. and PARKES, A. S. Seminal vesicles of the mouse	442
DOMENECH-ALSINA, F. Histamine hypotension	54
DOUGLAS, C. G., GREENE, C. R. and KERGIN, F. G. NH_4Cl and adaptation to low pressures	404
EULER, U. S. v. On "Novadrenine"	462
FRAZER, A. C. and WALSH, V. G. Fine emulsions and lipolytic rate	467
GARRY, R. C. Defæcation in the cat	208
GELFAN, S. and ADRIAN, E. D. Rhythmic discharges in muscle fibres	271
GOLDBLATT, M. W. Adrenaline and blood lactate	96
GRANIT, R. and CREED, R. S. Retinal action potential	419
GREENE, C. R., KERGIN, F. G. and DOUGLAS, C. G. NH_4Cl and adaptation to low pressures	404
HERTZ, D. H. and CREED, R. S. Strychnine and respiratory reflexes	85
HORNE, E. A., MAGEE, H. E. and BELL, D. J. The decerebrate rat	196
HORNE, E. A. and MAGEE, H. E. Glycogen synthesis in the small intestine	288
KERGIN, F. G., DOUGLAS, C. G. and GREENE, C. R. NH_4Cl and adaptation to low pressures	404

LIST OF AUTHORS.

vii

	PAGE
LAPIQUE, L. Slow muscle excitability curves	381
LUNDGAARD, E. and CATTELL, MCK. Isolated muscle and aerobic activity	246
MAGEE, H. E., BELL, D. J. and HORNE, E. A. The decerebrate rat	196
MAGEE, H. E. and HORNE, E. A. Glycogen synthesis in the small intestine	288
MATTHEWS, B. H. C. Nerve endings in mammalian muscle . . .	1
MAWSON, C. A. Lactate and creatinephosphoric acid	295
NEWTON, W. H. and SMIRK, F. H. Intravenous water and diuresis	451
PARKES, A. S. and DEANESLY, R. Hysterectomy and œstrous cycle	80
PARKES, A. S. and DEANESLY, R. Subcutaneous absorption of oils	155
PARKES, A. S. and DEANESLY, R. Seminal vesicles of the mouse .	442
PARKINSON, J. L. Form of muscle twitch	106
REID, C. and BANERJI, H. Adrenals and anæsthetic hyperglycæmia	370
RIDOUT, J. H. and BEST, C. H. Choline and liver fat with cholesterol	415
RITCHIE, A. D. Theories of muscular contraction	322
ROBSON, J. M. Reactivity of uterine muscle in pregnancy . .	309
SMIRK, F. H. Water absorption in man	113
SMIRK, F. H. Blood composition and diuresis	127
SMIRK, F. H. Water absorption in man	147
SMIRK, F. H. and NEWTON, W. H. Intravenous water and diuresis	451
STAMMERS, A. D. Leucocytes at high altitudes	335
TIEGS, O. W. and CORKILL, A. B. Sympathetic nerve and skeletal muscle	161
TOWER, S. S. Action potentials in sympathetic nerves	225
WALSH, V. G. and FRAZER, A. C. Fine emulsions and lipolytic rate	467
WHITTAKER, S. R. F. and WINTON, F. R. Viscosity of blood . .	339
WINTON, F. R. and WHITTAKER, S. R. F. Viscosity of blood . .	339

PROCEEDINGS OF THE PHYSIOLOGICAL SOCIETY.

February 18, 1933.

	PAGE
<i>Burns, Clarice Margaret.</i> Calcium salts of bone	1 <i>P</i>
<i>Magee, H. E. and Reid, Eric.</i> Indirect calorimetry applied to the fowl	2 <i>P</i>
<i>Pritchard, E. A. Black.</i> The occurrence of Wedensky inhibition in myasthenia gravis	3 <i>P</i>
<i>Hilton, R.</i> A mouthpiece for collecting expired air in dyspnoea .	5 <i>P</i>
<i>Knight, B. C. J. G. and Fildes, Paul.</i> An essential growth factor for the anaerobe <i>B. sporogenes</i>	6 <i>P</i>
<i>Harris, D. T.</i> Electrophoresis apparatus	8 <i>P</i>
<i>Winton, F. R.</i> Intrarenal pressure	9 <i>P</i>
<i>Brunton, Charles E. and Israëls, M. C. G.</i> Stethographic method to ensure that alveolar air samples are taken in the correct phase of respiration	11 <i>P</i>
<i>Teorell, Torsten.</i> Remarks concerning ionic distribution during mem- brane diffusion	11 <i>P</i>

March 18, 1933.

<i>Folley, S. J. and Peskett, G. L.</i> Automatic pressure control . . .	13 <i>P</i>
<i>Bickford, R. G. and Winton, F. R.</i> The influence of temperature on the isolated mammalian kidney	14 <i>P</i>
<i>Moncrieff, A. A. and Wilson, A. T.</i> A simple alcohol check apparatus for closed circuit metabolic work	16 <i>P</i>
<i>Theobald, G. W. and White, M.</i> An anti-diuretic substance extracted from the liver	18 <i>P</i>
<i>Marrian, G. F. and Newton, W. H.</i> Further observations upon the action of cestrin on the isolated uterus	19 <i>P</i>

May 13, 1933.

<i>Stammers, A. Dighton</i> Oxygen content, capacity and percentage saturation in arterial blood at Johannesburg (altitude 5750 feet)	21 <i>P</i>
<i>Matthews, Bryan H. C.</i> Human electrocardiograms recorded with the cathode ray oscillograph	21 <i>P</i>
<i>Thornton, J. W. and White, E. G.</i> Some estimations on the blood and urine of the 1933 Cambridge boat-race crew	23 <i>P</i>

June 10, 1933.

<i>Huggett, A. St G. and Rowe, F. M.</i> Azo-dyes as anti-coagulants .	25 <i>P</i>
<i>Lennox, J. A. (introduced by Burns, D.).</i> The effect of the removal of the parathyroid glands in dogs upon the gastric motility and secretions	26 <i>P</i>
<i>McSwiney, B. A. and Spurrell, W. R.</i> The emptying time of the stomach for hypotonic meals	28 <i>P</i>

CONTENTS.

ix

	PAGE
<i>Dickinson, S., Havard, R. E. and Platt, B. S.</i> The measurement of the hydrogen ion concentration of blood by the glass electrode	28 P
<i>Garry, R. C.</i> Reflex interdependence of the internal and external anal sphincters	30 P
<i>Robson, J. M.</i> The effects of certain tissue extracts on pregnancy	31 P
<i>McDowall, R. J. S.</i> Depressor shock	32 P

LIST OF AUTHORS.

BICKFORD, R. G. and WINTON, F. R. Temperature on isolated kidney	14 P
BRUNTON, CHARLES E. and ISRAËLS, M. C. G. Stethographic method for air sampling	11 P
BURNS, CLARICE MARGARET. Calcium salts of bone	1 P
DICKINSON, S., HAVARD, R. E. and PLATT, B. S. Glass electrodes for pH of blood	28 P
FILDES, PAUL and KNIGHT, B. C. J. G. Growth factor for <i>B. sporogenes</i>	6 P
FOLLEY, S. J. and PESKETT, G. L. Automatic pressure control	13 P
GARRY, R. C. Anal sphincters reflexes	30 P
HARRIS, D. T. Electrophoresis apparatus	8 P
HAVARD, R. E., PLATT, B. S. and DICKINSON, S. Glass electrodes for pH of blood	28 P
HILTON, R. Mouthpiece for air samples	5 P
HUGGETT, A. St G. and ROWE, F. M. Azo-dyes as anti-coagulants	25 P
ISRAËLS, M. C. G. and BRUNTON, CHARLES E. Stethographic method for air sampling	11 P
KNIGHT, B. C. J. G. and FILDES, PAUL. Growth factor for <i>B. sporogenes</i>	6 P
LENNOX, J. A. Parathyroids and gastric functions	26 P
MCDOWALL, R. J. S. Depressor shock	32 P
MCSWINEY, B. A. and SPURRELL, W. R. Gastric emptying of hypotonic meals	28 P
MAGEE, H. E. and REID, ERIC. Indirect calorimetry of fowls	2 P
MARRIAN, G. F. and NEWTON, W. H. Action of oestrin on uterus	19 P
MATTHEWS, BRYAN H. C. Electrocardiograms	21 P
MONCRIEFF, A. A. and WILSON, A. T. Alcohol test for metabolic apparatus	16 P
NEWTON, W. H. and MARRIAN, G. F. Action of oestrin on uterus	19 P
PESKETT, G. L. and FOLLEY, S. J. Automatic pressure control	13 P
PLATT, B. S., DICKINSON, S. and HAVARD, R. E. Glass electrodes for pH of blood	28 P
PRITCHARD, E. A. BLACK. Wedensky inhibition in myasthenia gravis	3 P
REID, ERIC and MAGEE, H. E. Indirect calorimetry of fowls	2 P
ROBSON, J. M. Tissue extracts on pregnancy	31 P
ROWE, F. M. and HUGGETT, A. St G. Azo-dyes as anti-coagulants	25 P

	PAGE
SPURRELL, W. R. and MCSWINEY, B. A. Gastric emptying of hypotonic meals	28 <i>P</i>
STAMMERS, A. DIGHTON. Oxygen in blood at Johannesburg	21 <i>P</i>
TEORELL, TORSTEN. Membrane equilibrium	11 <i>P</i>
THEOBALD, G. W. and WHITE, M. Anti-diuretic substance from liver	18 <i>P</i>
THORNTON, J. W. and WHITE, E. G. Boat-race crew	23 <i>P</i>
WHITE, E. G. and THORNTON, J. W. Boat-race crew	23 <i>P</i>
WHITE, M. and THEOBALD, G. W. Anti-diuretic substance from liver	18 <i>P</i>
WILSON, A. T. and MONCRIEFF, A. A. Alcohol test for metabolic apparatus	16 <i>P</i>
WINTON, F. R. Intrarenal pressure	9 <i>P</i>
WINTON, F. R. and BICKFORD, R. G. Temperature on isolated kidney	14 <i>P</i>

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NERVE ENDINGS IN MAMMALIAN MUSCLE.

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SINCE Ruffini [1898] gave us his beautiful drawings of muscle spindles, and Sherrington [1894] showed that they were sensory end-organs, our knowledge of their function and importance has been steadily growing owing to the observations of physiologists in many parts of the world, and particularly to those of Sherrington and his co-workers.

Until recent years our knowledge of the behaviour of the sense organs in muscle was obtained by inference and indirect observations. The only direct observations on mammalian proprioceptors made hitherto are those of Forbes, Campbell and Williams [1924], and McCouch, Forbes and Rice [1928], who, using a string galvanometer, were able to show that sensory action currents occurred after a muscle had contracted. But now the methods pioneered by Adrian have made direct observation of the response of these nerve endings possible, and it has only been necessary to adapt the technique to the peculiarities of the problem for the behaviour of mammalian muscle spindles to be studied directly.

A considerable amount of work has already been done on the nerve endings in frog's muscle [Adrian and Zotterman, 1926*a*; Bronk, 1929*a* and *b*; Matthews, 1929*b*, 1931*a* and *b*], but the histology of the nerve endings in mammalian muscle is far more complex than that of the muscle spindles in the frog, and does not justify the assumption that they behave in exactly the same way.

The present work has been undertaken to see what can be learnt of these nerve endings by direct observation, and this paper is a general survey of the subject, as the work has raised many new problems which have not as yet been fully examined.

The technique employed is essentially the same as that by which muscle spindles in the frog were studied [Matthews, 1931*a*, *b*], but many modifications in details have been necessary.

In any study of nerve endings by the electrical method it is almost essential to work with single nerve endings in order to discover the finer details of their behaviour and avoid statistical effects. It is much more difficult to isolate single nerve endings in mammalian muscles than in those of the frog, for the frog most conveniently possesses a small muscle containing only one spindle, and this has only a single sensory nerve ending. In mammalian muscles not only are there a number of spindles each containing several sensory nerve endings, but other endings are present as well, *e.g.* the tendon endings, small fibres which end among the blood vessels, and endings in the fascia associated with the muscle. It has not been found possible to confine the stimulus to single nerve endings, and so it has been necessary to restrict the fibres under observation. This has been done by the method, first used by Adrian and Bronk [1928], of cutting through the nerve below the recording electrodes until only one sensory fibre remains intact, and several hundred single nerve endings have been examined by this means.

PART I. METHOD.

Electrical recording apparatus.

The oscillograph and amplifier which have been described in previous papers [Matthews, 1928, 1929*a*] were used for the present work; the oscillograph was fitted with a reed of natural period 6500 vibrations per sec. and was critically damped electromagnetically by short-circuited copper loops on the extremities of the pole pieces. The distortion of nerve action potentials recorded at body temperatures will be appreciable, but these distortions do not enter into most of the results considered in this paper and so may be neglected; as it is the spacing and frequency of the action potentials which are the principal physiological interest, their time relations here are at present of lesser importance.

Small coupling condensers ($0.001 \mu\text{F}$) were used in the amplifier in the way previously described [Matthews, 1931*b*] in order to allow of recording sensory action potentials a few thousandths of a second after the whole nerve trunk had been stimulated electrically. In some experiments larger coupling condensers ($0.25 \mu\text{F}$) were used when the shape of the action potential was examined, and in a few very large condensers were used ($4 \mu\text{F}$) to see if there was any evidence of the presence of very slow action potentials which might fail to be recorded when the small condensers were in use. The output of the second stage of the amplifier was also fed to a three-valve amplifier feeding a loud speaker.

Camera.

The two-record camera giving simultaneous records at 5–20 cm. per sec. and at 2–4 m. per sec., which has already been described [Matthews,

1931*a*], was used to record the deflections of the oscillograph, the myograph and the time marker. In some experiments a Cambridge Instrument Co. 59 mm. paper camera was used when the width of cine bromide paper was insufficient to accommodate the whole record. Its driving gear was removed and it was driven by a powerful (Electromphone) electric gramophone motor, working direct on to the paper roller shaft through a universal joint; this gave the paper speeds from 5 to 20 cm. per sec. A rotating mirror device, such as has been previously described [Matthews, 1929*b*], was used to observe deflections before and during photographic recording.

Myograph.

A special type of myograph was designed and made for this investigation in order to record tension, extension, speed of loading, and contraction of the muscles. It is illustrated in Fig. 1.

The whole was built up on a small steel girder to which the drills in the animal's leg were clamped by insulated bushes so that the girder could be earthed for shielding purposes without earthing the body of the animal. A sliding carriage (*B*) carried the myographs which were of the torsion spring type and also a spring which could be used as an inertialess load for isotonic recording. The principle of this is illustrated in the inset to Fig. 1. A clock-spring barrel was used; the spring could be wound to any desired tension and when the muscle shortened the load only rose by 1-2 p.c. As the weight of the moving parts was only a few grams, inertia difficulties were eliminated. The whole myograph carriage was arranged to slide on a 7/16 silver steel rod (*C*) and a flat guide plate (*D*); it moved between adjustable stops, and a range of movement of up to 6 cm. was available. The rate of movement of the carriage was controlled by a dashpot (*E*) operating through a bell crank; a screw-down steam valve (*J*) regulated the flow of fluid from below to above the piston. The carriage was driven forward by a plunger in a cylinder (*G*) connected to the compressed air supply (25 lb. per sq. in.); it was returned by a smaller piston and cylinder (*F*). The carriage could thus be moved forward, stopped and returned in any way required by manipulation of the air-supply, stops, and dashpot valve.

Light was directed on to the myograph mirrors from the same arc light that illuminated the oscillograph; it was arranged that the incident and reflected beams were exactly in the line of travel of the carriage, so that there was no shift of zero as the carriage travelled forward. The distance from the mirror to the camera was about 7 m., so that there was no appreciable change in magnification when the carriage travelled a centimetre, which was about the movement usually employed. The carriage was designed so that all the strains came under the centre line of the rod on which it slid, so that there was no tendency for it to slew to either side; had any unsteadiness occurred it would have been very apparent, as it would have been enormously magnified by the 7 m. beam of light; absolutely no trace of such unsteadiness could be detected as the carriage moved, which may be attributed to the central disposition of the strains and the very excellent fit of the carriage on its slides. The coarse vibrations in some of the tension records below are due to the vibration of the bench on which the myograph was mounted. The tension myograph carried two mirrors, one near the end and a second giving five times as much magnification near its centre; this was found a great convenience, as small tensions applied to a muscle could be read with

accuracy from the sensitive mirror while the less sensitive one could record large tensions (e.g. during tetanic contraction) after the other beam had deflected right off the camera slit. A third mirror recorded the movement of the arm of the isotonic spring load; if a thread was attached to this arm and to a pin on the main girder this mirror could be used to record movement of the carriage and thus extension of the muscle.

With this myograph the muscle can be stretched or released by any amount at any desired rate or loaded or unloaded at any rate. Moreover, every mechanical change in the muscle is recorded on the film, with the coincident electrical record.

Stimulating arrangements.

For electrical stimulation of the nerve two methods were used, a coreless induction coil with close coupled coils and a calibrated rheostat in the primary circuit, and a neon lamp apparatus. The latter was found extremely convenient, as the rate of stimulation could be varied instantly by altering the capacity across the lamp and the charging resistance, and the strength varied by a potentiometer in series with the lamp. Either stimulating circuit could be connected to the Ag-AgCl type stimulating electrodes through 0.001 farad condensers to prevent any steady current leakage and to abbreviate the stimulus escape.

Material.

Cats were used in all the experiments described here. Guinea-pigs were used in a few preliminary experiments, but were not found to be very suitable owing to their small size and frailty.

In most experiments the animals were anaesthetized with chloroform and ether and decerebrated, at first with a Sherrington frame, in later experiments by trephining; the sciatic and femoral nerves and the attachment of psoas were cut under anaesthetic to immobilize the limbs. In some early experiments urethane (1 g. per kg.) was used, but it was discontinued as it was found to affect the response of the nerve endings; this will be discussed in detail later. Spinal animals were not found to be very satisfactory owing to the low blood-pressure and difficulty of correct ventilation; any deviation from normal in these respects was found to alter the response of the nerve endings.

Shielding, etc.

The animal was placed on a wooden stand in a large earthed iron box to which the myograph was bolted. The box had a detachable plate-glass front and served both for shielding the preparation from electric and magnetic disturbances and also for warming; the floor was heated with a gas ring and was flooded with water to keep the air moist. In some experiments the nerve was enclosed in a moist chamber, obviating the

necessity of flooding the box. The trachea tube was arranged so that the animal could either breathe cold air from outside the box or warm air from within. Temperatures were observed with a needle thermocouple connected to a galvanometer recalibrated in ° C.; the cold junction was housed in a thermos flask. Thus the body temperature of the cat could be observed, or that of the muscle under observation only, by inserting the needle under the skin close to the muscle. An electrically heated pad was sometimes used to raise the temperature of a limb; it was supplied from an accumulator, one pole of which was earthed to avoid bringing electrical disturbances into the animal box. Steel hooks were tied into the tendons and connected to the myograph with steel wire.

Isolation of single nerve endings.

Various methods of isolating the response of single nerve endings were tried. It seems unlikely that muscles exist in the cat which contain only one sensory ending such as were found in the frog's toes [Matthews, 1931*a*], though muscles have been described containing two or three muscle spindles [Hines and Towers, 1928]. It was therefore necessary to take a muscle containing a number of nerve endings and put all but one out of action. Preliminary experiments in which the muscle was divided showed that it was possible to isolate single endings in this way (which was used by Adrian and Zotterman [1926*a*] in their first isolation of a single nerve ending in frog's muscle), but in cats the endings obtained in this way rapidly deteriorated and soon failed altogether. Perhaps their failure was in part due to liberation of harmful substances in the muscles, but the chief factor was probably the almost inevitable failure of their blood supply resulting from cutting the muscle, for later experiments have shown that the behaviour of nerve endings in muscle is modified by circulatory arrest, and the endings fail a short time after the blood supply of a muscle is cut off. This method was therefore abandoned.

Another method which was tried was that of blocking conduction in the nerve below the recording electrodes by means of a constant current passing between a second pair of electrodes, for I have found in experiments on the frog (unpublished) that by minutely controlling the blocking current conduction can be suppressed in all but one sensory fibre, so that the response of a single ending can thus be followed in a preparation containing a number of nerve endings. However, when this was tried on cat's nerve it was found that the steady blocking current provoked a random discharge of impulses in the nerve and often started rapid discharges of the type described by Adrian [1930] and shown by him to originate in

damaged fibres or endings. Also it is impossible to be sure that the one sensory fibre which is not blocked is conducting normally and transmitting all the impulses which come to the polarized region. This method was therefore also abandoned in favour of section of the nerve between the muscle and recording electrodes. This method, which was used by Adrian and Bronk [1928] to isolate the response from a single motor nerve cell, and by Bronk [1929*a*] to isolate endings in frog's muscle, does not suffer from any of the disadvantages of the methods discussed above. It only presents difficulty in the dissection of the nerve, but after some practice it is possible to make a successful single nerve ending preparation at nearly every attempt, and more than two hundred have been made in the course of the present work.

The dissection of the nerve is carried out on a black glass plate under strong light with a low-power dissecting binocular, using needles sharpened to a cutting edge on an oil-stone; the nerve sheath is removed for a short distance and the nerve separated into bundles of fibres; these are cut in turn and after each section the upper part of the nerve is placed on electrodes and the appropriate muscle is stretched. From the sound of the amplified action currents in the loud speaker it is possible to tell by ear how the dissection is proceeding. When only the last bundle remains unsevered the response has as a rule resolved itself into a countable number of superimposed rhythmic discharges, and redivision and section of the remaining bundle is carried on until only a single rhythmic response results on stretching the muscle. If a slip is made and all response is abolished the section can be repeated lower down if a long stretch of nerve is available.

The nerve-section method of isolating nerve endings selects one of the endings present entirely at random; if a number of different types of ending are present one type is selected by chance in each experiment. Moreover, if a large number of preparations are made the endings selected by section should indicate all the types present, and the relative frequency with which each type is met with will roughly indicate the proportion in which the various types of ending occur in the muscle. Actually four types of behaviour have been met with in single-ending preparations.

When the nerve is divided into its natural components it is necessary to remove as much as possible of the connective tissue from the nerve bundles, for if this be left on the nerve in a damaged state discharges of the type described by Adrian [1930] coming from endings associated with the nerve sheath and blood vessels occur and somewhat mask the impulses from the endings in the muscle. Fortunately they can be distinguished from the latter, as they are quite unconnected with stretching of the muscle, and to avoid them most of the nerve sheath is stripped off.

The responses in the nerves of a number of muscles have been examined (sartorius, gastrocnemius, tibialis anticus, peroneus longus, peroneus brevis, soleus, and various toe muscles). The most detailed study has so far been carried out on peroneus longus and soleus, and sufficient results are to hand to form some idea of the behaviour and relative abundance of the different types of nerve ending in these two muscles. The results from other muscles do not show any obvious difference from these two muscles.

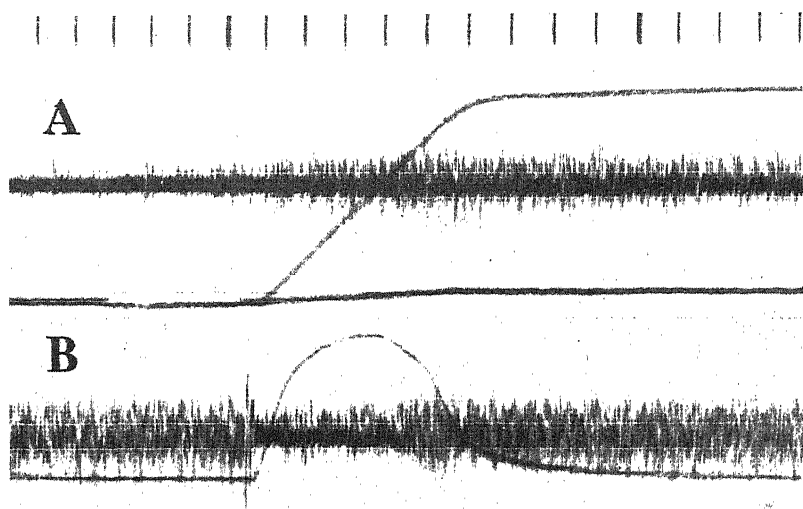


Fig. 2. Response from whole nerve to soleus. A, on stretching, cross-line signals extension, bottom line tension (final value about 10 g.). Centre oscillogram. B, during isotonic twitch, tension 50 g. Time marker at top 0.05 sec. intervals. This and all subsequent figures read left to right and are about half the size of the original records.

Results.

If a muscular division of either the peroneal or popliteal nerve be placed on the electrodes while the muscle attachments are intact, there is an irregular discharge of impulses continually in progress. This discharge originates in the muscle proprioceptors, for it is greatly reduced if the tendon of the appropriate muscle be freed from its attachments. The slight residual response when the muscle is quite free ceases for a few seconds if the muscle be stretched and released, and then gradually recommences. It thus appears that a number of the endings in muscle set up a resting discharge in the absence of stimulation, but later it will be

shown that they do not all do so. If the muscle be stretched there is an enormous sensory response and the action potentials from so many fibres are superimposed that analysis is impossible.

When the muscle is stretched and kept under tension, even of only a few grams, a large irregular discharge of impulses occurs in its nerve, and this continues indefinitely as long as the tension is maintained. This is illustrated in Fig. 2 A, which shows the response from soleus, and Fig. 2 B shows the response when the muscle contracts in response to a maximal stimulus applied to the nerve below the recording electrodes (by a second pair of electrodes). It is at once clear from the complexity of the response that the details of the behaviour of these end-organs can only be learnt by studying them singly.

It has been mentioned above that four types of behaviour have been met with in single-ending preparations. The four types of behaviour have been designated A 1, A 2, B and C for convenience in reference and will be described successively. The distinction of these types of behaviour is based not only on the response of the preparations to passive stretch but also on what happens to the response during active contraction of the muscle; as will be seen the different types of behaviour are totally distinct and readily identified.

PART II. THE TYPES OF RECEPTOR PRESENT.

A 1 type of behaviour.

This type of response is that most frequently met with and has been found in every muscle that has been examined; about 50 p.c. of all the endings isolated have been of this type. The behaviour of these receptors is very like that of the frog's muscle spindles.

Response to stretch.

The endings giving the A 1 response have a very low threshold, and the least tension on the tendon, even 1 or 2 g., is sufficient to evoke a rhythmic discharge of impulses from them; in addition, in a number of preparations a regular discharge (5-15 per sec.) is present in the absence of external tension, but this ceases entirely for a few seconds after the muscle has been stretched and released, and then gradually builds up to its old rate again. But in about two-thirds of the preparations this resting discharge is absent. When present, in many preparations one or two impulses are set up at each heart beat, and these vanish instantly when the arteries are clamped, but more often the resting discharge rate does not

correlate with the heart beat. The receptors in mammalian muscle differ very markedly from those in the frog, in that they are able to set up discharges which are regular at very low frequencies. In the frog at 15° C. regular discharges below 15 per sec. rarely occur, discharges below this frequency being a random succession of impulses. These mammalian endings, however, often set up regular discharges at rates as low as 5 per sec., and the spacing of the impulses does not differ from the mean by 10 p.c.; irregular discharges above 10 per sec. have rarely been found. After

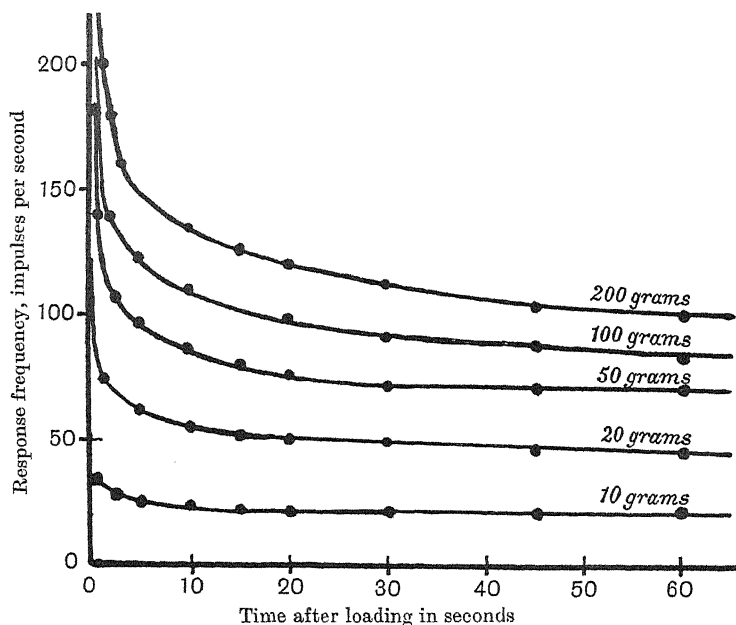


Fig. 3. Graphs of response to various tensions.

one of these endings has been subjected to a fairly big (250 g.) stretch for some minutes, its threshold on unloading is found to have risen considerably, for if thereafter it be restretched very gradually a higher tension is reached before it starts to respond than is the case when it is stretched without such previous loading; however, when the response starts it has about the same rate as the starting rate in the first case. The response frequency at any tension is slightly lowered if that tension immediately follows a previous stretch; this effect is most evident at low tensions. The effects of previous stretch on the response to subsequent restretch have not been worked out quantitatively on the endings in mammalian muscle,

but this has been done with the endings in frog's muscle [Matthews, 1931*a*], and the present observations suggest that here also the end-organ takes some seconds to return to its resting condition after it has been responding.

In Fig. 3 the response of one of these endings when various loads are hung on the tendon is shown graphically. Over this range the frequencies

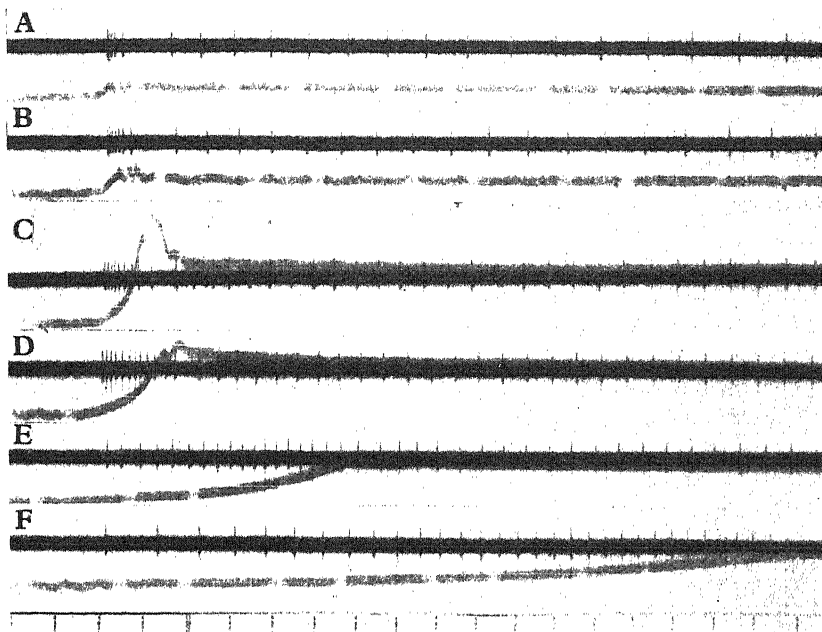


Fig. 4. Records of response from a single nerve ending (A 1), in peroneus longus. Electrical record and record of tension. A, B, C, stretch at 20 mm. per sec. to final tensions of 10, 15, 25 g. C, D, E, F, stretch at various rates to a final tension of 25 g. Time marker at bottom 1/20 sec.

set up are approximately proportional to the logarithm of the load on the tendon, as was found to be the case with frog's muscle spindles; at high tensions the response falls short of this proportionality. If the logarithm of load-frequency relationship be plotted the slope of the lines from different preparations is by no means the same. The graphs in Fig. 3 only cover the first minute after loading, but the end-organ continues to respond at a rate which declines more and more gradually, until after about an hour it becomes steady, and there seems no reason to suppose it would not continue thus indefinitely. In a few preparations the response has continued

to fall and ceased after a few minutes' loading, but the majority have continued to respond in the way described above. One preparation of a receptor in one of the interossei muscles of the cat's foot ceased to respond about 30 sec. after loading. With large loads of 500 g. and up-

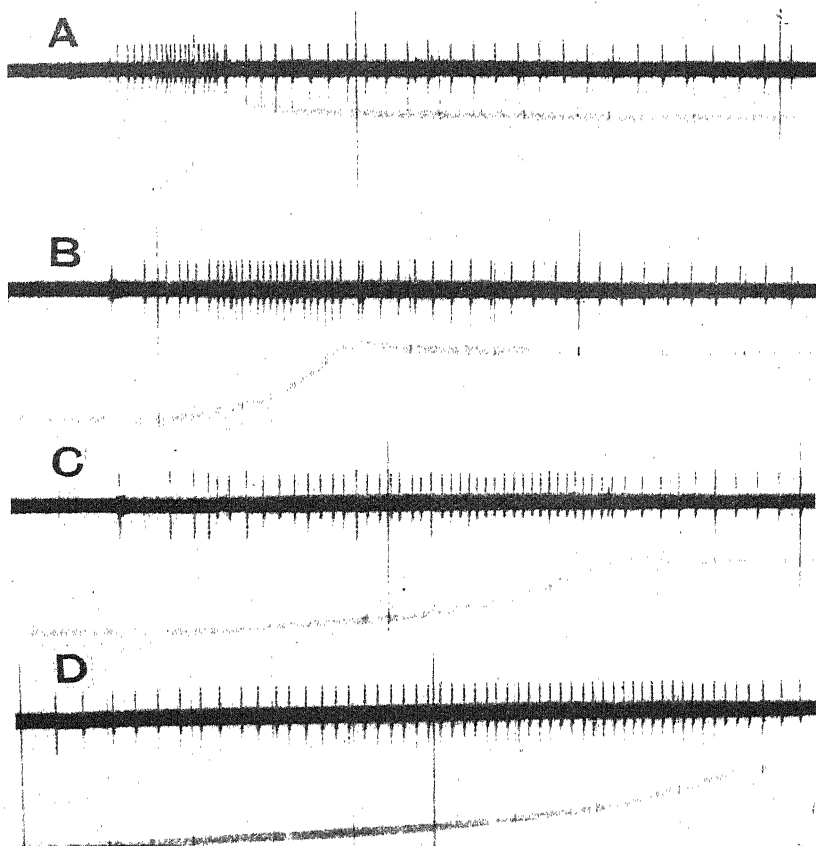


Fig. 5. Records of response of a single nerve ending (A 1) in soleus. Stretch to a final tension of 55 g. at rates indicated by the tension record. Cross-lines 1/10 sec.

wards the response may cease abruptly or undergo a transient acceleration a minute or two after loading, but it is thought that this may be due to the effects of constriction of the circulation rather than to adaptation (discussion of this phenomenon will be deferred to a later section of this paper, where the effects of circulatory arrest are considered).

Extension.

So far the response of the end-organ to steady tension only has been considered. It is found that during extension the end-organ responds at a high rate. In Fig. 4 C, D, E, F are shown records of the response when peroneus longus is extended by a fixed amount at various rates; Fig. 5 shows similar records from an ending in soleus, and in Fig. 6 these records are presented graphically.

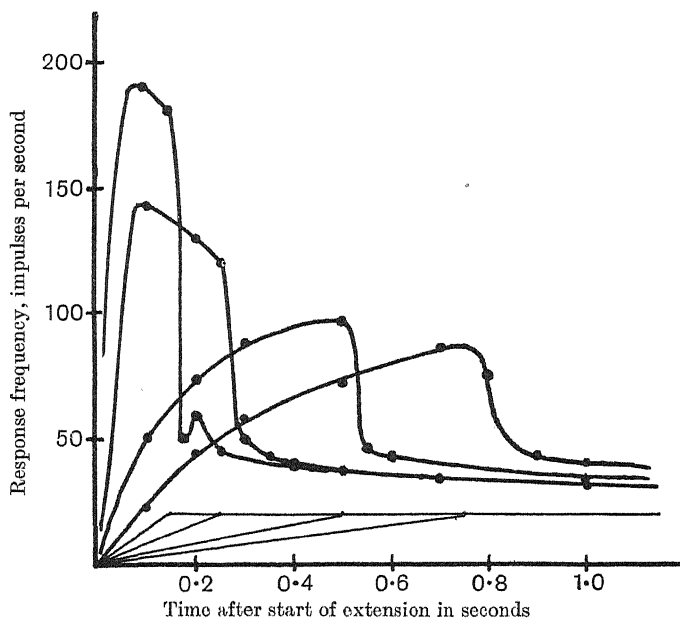


Fig. 6. Graphs of the response of an ending (A 1) in soleus. Stretched at rates indicated at the foot, to a final tension of 65 g.

If the stretch is very rapid the maximum frequency occurs during the stretch before the maximum tension has been reached (see Figs. 4 C, 5 A); the reason for this may be that with the very high frequencies that are set up adaptation is so rapid that by the time the maximum tension is reached considerable adaptation has already occurred. But several other possible explanations must be considered. It is found that the maximum frequency set up depends much more on the rapidity of stretching than on the final value of the tension; a slight stretch applied rapidly can evoke a very high initial frequency of response (see Fig. 4 A, B). This suggests

that during rapid stretch the stimulus to the nerve ending may rise far above its later steady value; this may occur on account of the mechanical properties of the end-organ.

Discussion of viscous effects.

In a previous paper in which similar effects were recorded from frog's muscle spindles it was suggested that the high initial frequency and rapid fall of frequency that occurred on rapid stretch might be due to the

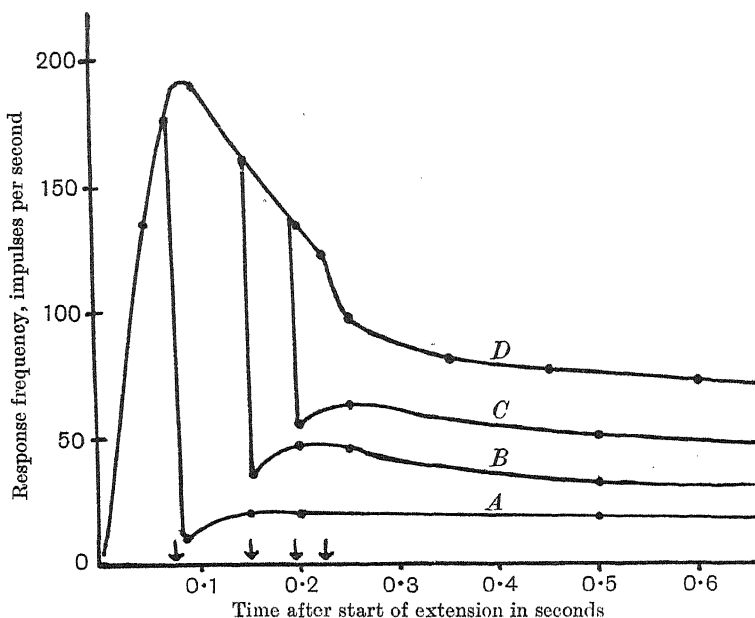


Fig. 7. Graphs of an A1 ending in soleus stretched at a fixed rate to final tensions of 20, 50, 100, 200 g. The arrows indicate the conclusion of extension in these four cases.

terminal portions of the structure on which the nerve ending was situated being more viscous than the centre; or alternatively, that the whole end-organ structure might be more viscous than the surrounding muscle fibres, so that during rapid stretch the deformation of the nerve ending would be much greater than after a steady extension had been reached. Whatever the arrangements may be the forces acting on a viscous elastic end-organ will be greater during extension than after equilibrium is reached in a new position. It will be seen below that the viscous elastic interpretation accounts very well for the observed behaviour of the cat's receptors, and support of the view that the end-organ is more viscous than

the surrounding muscle fibres comes from observations to be described later of the behaviour of the endings during active shortening of the muscle.

In Fig. 7 are shown graphs of the response when the muscle is extended by various amounts at a fixed rapid rate. When extension ceases (Fig. 7 A, B, C) the response does not fall smoothly to a lower level, but it abruptly falls below it and then rises up to it. This strongly supports the view that during stretch overloading of the end-organ is occurring owing

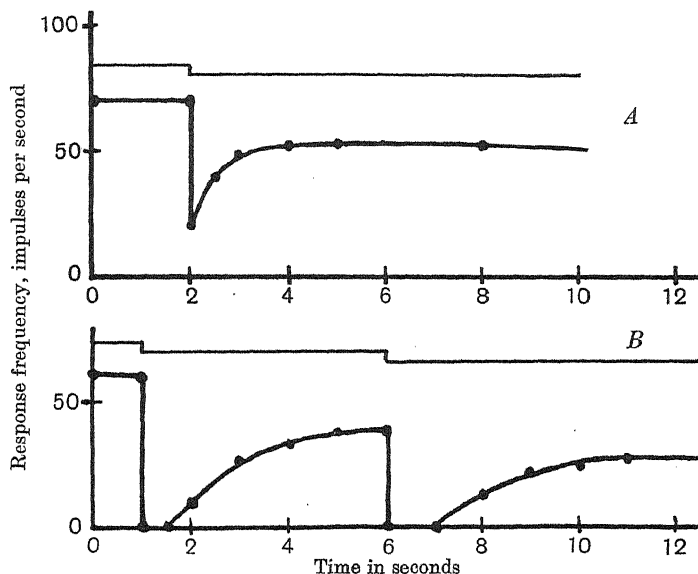


Fig. 8. Graphs of response on quick release. A, type B endings in soleus (to be discussed later in text). Tension 350 g. falls to 215 on release of 1 mm. B, type A 1 ending in soleus. Tension 160 g. falls to 95 and 60 g. on two successive releases of 1 mm.

to viscous forces. During stretch the stimulus to the end-organ would reach a value determined by the relative viscous elastic properties of the end-organ and surrounding muscle fibres; when extension ceases the strains on the end-organ will fall to those determined by the elasticity of the tissues. Thus at the cessation of extension the stimulus to the end-organ will fall to a lower value, causing a fall in the response which will then rise again as the adaptation caused by the previous greater stimulus and response passes off.

Quick release.

If a steady tension on the muscle be suddenly reduced to a steady lower tension the response stops and slowly rises up to the new lower

frequency. This is shown in Fig. 8 B from an ending in soleus. This again indicates that the end-organ may behave as a viscous elastic structure. The rise to the new frequency occurs much more slowly than the return of tension in the whole muscle, and though part of this rise may be due to adaptation produced by the previous greater stretch passing off gradually, it certainly accords with the view that the end-organ is more viscous than the surrounding muscle fibres. The rise noted above, and ascribed to disappearance of adaptation in Fig. 7 A, B, C, occurs much more rapidly than the rise after quick release.

In Fig. 7 D it will be seen that 0.1 sec. after the beginning of extension the response rate begins to fall although the tension is rising and extension still occurring; this fall in rate is probably partly due to adaptation on the part of the nerve ending.

Theoretical view of viscous effects.

The fall in response which occurs at the end of extension is much more marked when the final tension reached is small (Fig. 7 A) than when it is considerable; if viscous overloading occurs during stretch this is to be anticipated for the following reasons. The overloading stress in the end-organ due to its viscosity may be defined as the amount by which the force necessary to extend the end-organ by a small amount at a given rate exceeds that necessary to extend it the same amount infinitely slowly. Thus during extension the stress in the end-organ will be (a), the tension due to its elasticity, + (b), the overloading stress due to viscosity, which will depend on the rate at which it is being extended. When the rate of stretch is fixed b is constant, while a increases with the extension (though not in direct proportion). Thus as the extension increases the viscous overloading stresses become a smaller and smaller fraction of the total stresses in the end-organ; as a result the disappearance of (b) at the end of extension should have less and less effect on the response as the extension increases. Secondly, as the response rate varies as the logarithm of the steady tension on the muscle and therefore presumably roughly as the logarithm of the tension on the end-organ, large percentage changes in this tension will have far more effect on the rate of response at low than at high tensions.

From the above arguments it is clear that the initial overloading effect due to the viscosity of the end-organ should be most in evidence when the tension is small and the extension rapid, which is indeed the case (see Fig. 4 A).

Comparative rate of stretch.

If the records from soleus and peroneus longus be compared (Figs. 4 and 5) an interesting difference may be seen in the actual speed of stretch necessary to bring the rapid initial response into prominence in these two muscles. In Figs. 4 E and 5 B the rate of stretch is about the same, yet the ending in soleus responds twice as fast during stretch as it does later at constant length, while that in peroneus longus responds hardly any faster during stretch than subsequently. To obtain the same relative acceleration of response during stretch a much more rapid stretch must be given to peroneus longus than to soleus; the end-organs in soleus thus appear to be much more viscous than those in peroneus longus. It seems likely that the differences in viscous elastic properties of red and white muscle exist also in the intrafusal fibres of their muscle spindles, for evidence will be presented later which suggests that the A1 type of response comes from nerve endings in the muscle spindle.

Other possible explanations of these phenomena.

The records obtained by Hartline [1932] of the impulses set up by single receptors in the eye of *Limulus* when suddenly illuminated look very like those obtained here by sudden slight extension of a muscle. As in Hartline's preparation there are no mechanical factors involved, it does not seem impossible that the abrupt fall in response to a sudden stimulus might be partly due to some fundamental property of nerve endings, such that they are able to respond at a high rate initially to a sudden stimulus but shortly fall suddenly to a much lower level of activity, although the stimulus is still present at full intensity. The possibility of something of this sort occurring cannot be entirely neglected, but it is very unlikely that the phenomena studied here are due to such properties of the nerve ending, as they seem to be very closely connected with the mechanical events in the muscle and end-organ and they do not occur with the B endings described below; though by the present methods it is impossible to dissociate completely the effects on the response of adaptation and yielding of viscous elastic structures, it is probable that both factors are of importance, each predominating under particular conditions. The peculiar features of the response of these endings seem to be due mainly to mechanical factors.

The records of Fig. 4 A and B bear a superficial resemblance to those obtained by Tsai [1932] when he recorded the response from a suddenly stimulated receptor (frog's muscle spindle) above a region of impaired conduction; the pause in the response here cannot be due to impulses being blocked somewhere on their way to the recording electrodes, for this pause can be abolished by altering the stretch applied to the muscle.

Maximum rate of response.

The highest frequency of impulses that has been recorded from these endings is about 500 per sec. The absolute refractory period of the fibres supplying these nerve endings would allow of somewhat higher frequencies being transmitted, for these frequencies can still be recorded when the temperature of the nerve is lowered to about 34° C., though if it

is lowered below this partial blocking of the response occurs. It follows that normally the limit is set by the nerve ending rather than the nerve fibre, as Adrian has shown to be the case for a number of types of receptor. However, such frequencies can only be evoked by extremely violent stretch (5 mm. in 1/50 sec.); it does not seem likely that such sudden stretch could ever occur in the intact animal.

Response during active contraction.

The A1 type endings do not respond during active contraction of the muscle, and in this their behaviour closely resembles that of the frog's muscle spindle.

If the muscle is not subjected to any initial tension there is no response during contraction (see Fig. 9 F). If the muscle is subjected to initial stretch the response already in progress ceases during the rising phase of the mechanical response and restarts as the muscle relaxes. The behaviour of the A1 endings is illustrated in Fig. 9, which shows the cessation of the response during isometric twitch A and B in peroneus longus, C and D in soleus, at low and high initial tension. In Fig. 9 C and D two impulses appear during the twitch; this has been seen in a few preparations, but usually the pause in the response is absolute. If shortening is allowed to occur the pause in the response takes place in the same way (see Fig. 9 E).

During isometric tetanic contraction the response ceases and restarts on relaxation at a slightly higher rate; this is illustrated in Pl. I, Fig. 10, A is recorded from an ending in peroneus longus and C from one in soleus. If the initial tension is considerable the response may not cease entirely during isometric tetanic contraction, but its rate falls considerably (see Pl. I, Fig. 10 D). When the muscle is tetanized for some seconds the response often gradually reappears at a greatly reduced rate. The response stops completely during tetanus if shortening be allowed (Pl. I, Fig. 10 B), and when the muscle relaxes after isotonic tetanic contraction a burst of impulses is set up as relaxation occurs which is very like that recorded when the muscle is suddenly stretched (cf. Fig. 4 B).

Strength of stimulus.

In all the cases considered above the stimulus was at least 30 p.c. supramaximal. If the stimulus be gradually reduced the pause during twitch becomes less marked as the mechanical response becomes smaller, and disappears with the twitch. If the stimulus be reduced during tetanus the response reappears as the tension falls, and when the stimulus becomes sub-threshold the response returns to that determined by the

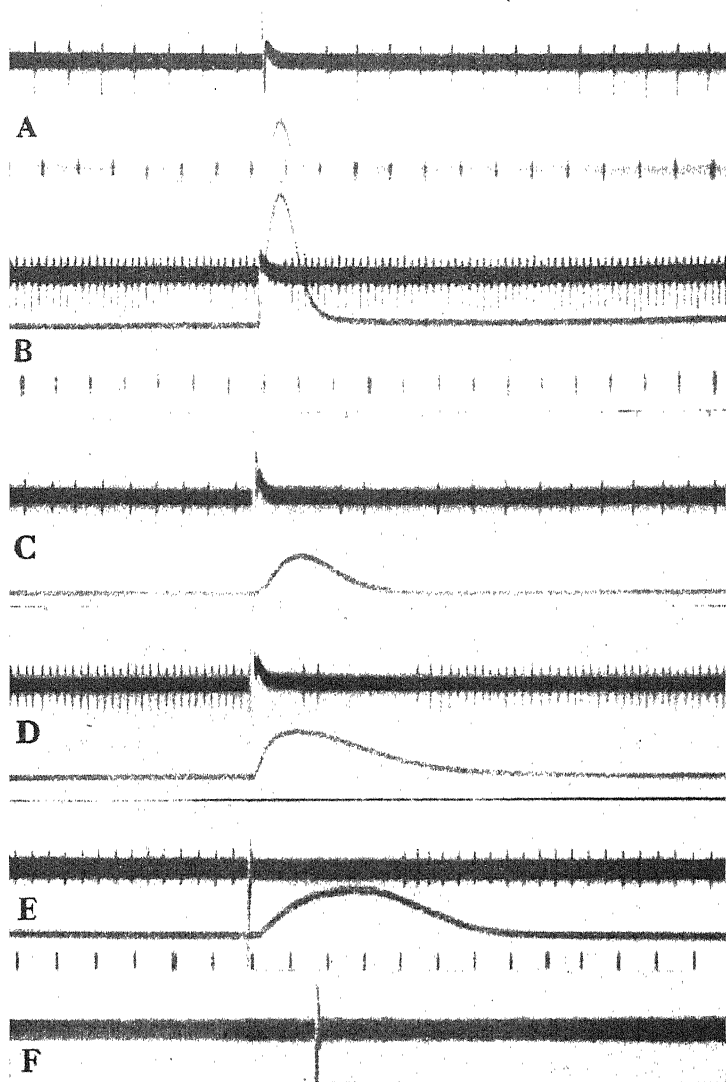


Fig. 9. Response of A1 endings during twitch. A, B, peroneus longus. Maximal isometric twitch, initial tensions 10 and 200 g. C, D, soleus maximal isometric twitch, initial tensions 10 and 120 g. E, soleus, maximal isotonic twitch, tension 40 g. F, soleus maximal twitch tendon free. Time marker 1/20 sec.

stretch background. The rate of tetanization is not found to have any significant effect on the response except in so far as it affects the mechanical response. The rate is usually kept as low as will give nearly complete fusion, as higher rates confuse the record with frequent stimulus escapes.

Interpretation.

All the above observations seem to allow of only one interpretation, that these stretch receptors lie "in parallel" with the contractile elements which when they contract take the strain off the end-organ, and cause its response to cease. That this occurred in muscle receptors was suggested by Fulton and Pi-Suner [1928] to account for the silent period in mammalian reflexes. Hoffman [1919] found that when a muscle exhibiting the stretch reflex contracts in response to reflex or motor stimulation there is a cessation of action currents in the muscle during the contraction; this phenomena has since been studied in detail by the above authors and by Denny Brown [1928].

The view that the receptor lies "in parallel" with the muscle fibres was found when modified to account entirely for the behaviour of the frog's muscle spindle [Matthews, 1931*b*], but to account for the pause observed to occur in the response from the frog's muscle spindle during isometric contraction, it was necessary to assume that some internal shortening took place, which it was suggested occurred owing to the elastic yielding of the muscle extremities. This elastic yield assumed to be present has now been demonstrated by Eccles (personal communication), who finds that a tension of 200 g. will produce an elongation of about 1 p.c. in the tendon of a cat's soleus muscles.

Thus during twitch in soleus an elongation of the order 1-2 mm. is to be expected in its tendon. In Fig. 8 B is shown a graph of the fall in the response that occurs when a stretched muscle is suddenly released 1 mm.; the response stops, and only after some seconds begins to rise up to a new lower level. Thus the shortening allowed by the yielding of the tendon may well contribute to the cessation or reduction of the response which occurs during isometric contraction. If the initial tension is greater than that which the muscle can produce, there is no pause in the response when the muscle twitches and only a slight reduction in its frequency; under these conditions the action current and chemical changes are still taking place in the muscle, so these cannot be held responsible for the pause, which seems only to be associated with the mechanical changes in the muscle. If the A1 type receptors lie in the muscle spindles their behaviour is entirely accounted for by the

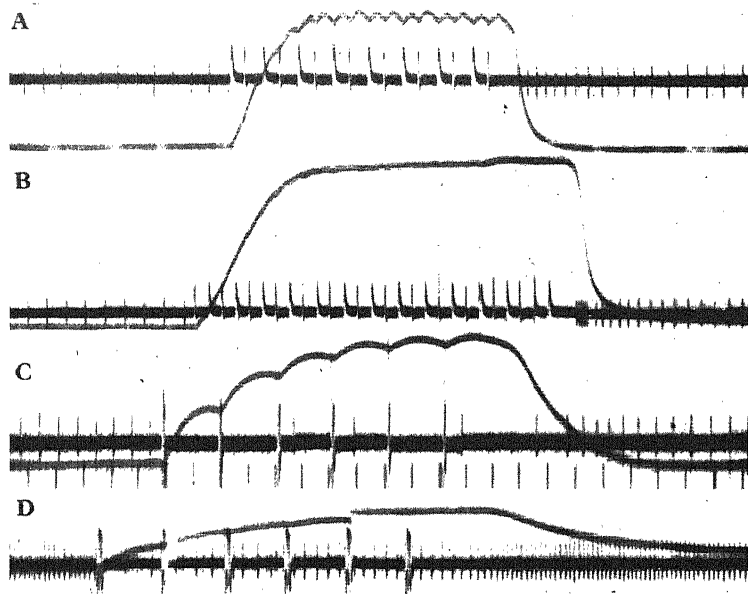


Fig. 10. Response of A1 endings during tetanic contraction. A, peroneus longus. Maximal isometric tetanus. Initial tension 20 g. B, peroneus longus. Maximal isotonic tetanus. Tension 25 g. C, D, soleus maximal isometric tetanus, initial tensions 10 and 500 g. Time marker 1/20 sec. at foot of C.

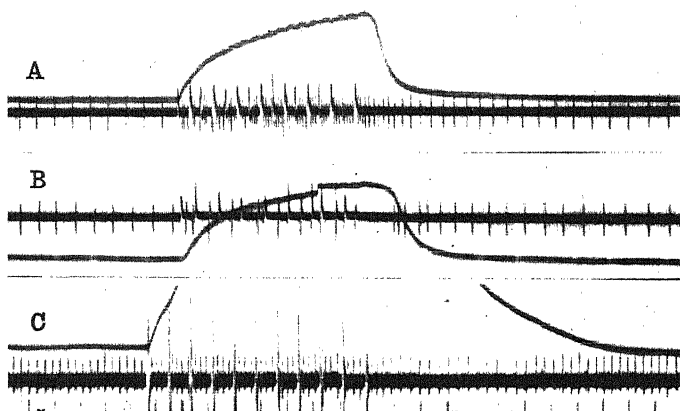


Fig. 11. Response of A2 endings. A, B, peroneus longus, both from the same ending, initial tension 25 g. Stimulation in A maximal, in B 85 p.c. of maximal. C, soleus stimulation 110 p.c. maximal, initial tension 500 g. Time marker 1/20 sec. at bottom.

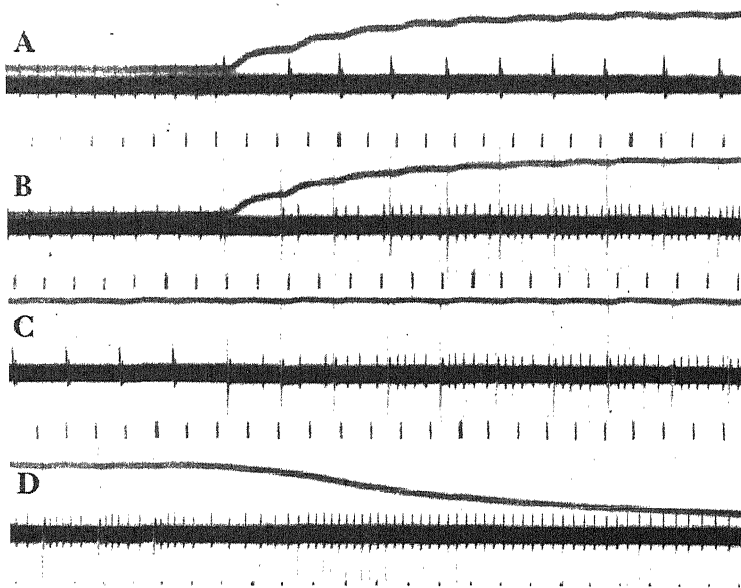


Fig. 12. Response of an A 2 ending in soleus during isometric contraction, initial tension 100 g. A, start of maximal tetanic stimulation. B, start of 110 p.c. maximal stimulation. C, tetanic stimulation; after the fourth stimulus escape the stimulus is suddenly increased from maximal to 110 p.c. maximal. D, conclusion of 110 p.c. maximal stimulation. Time marker 1/20 sec.

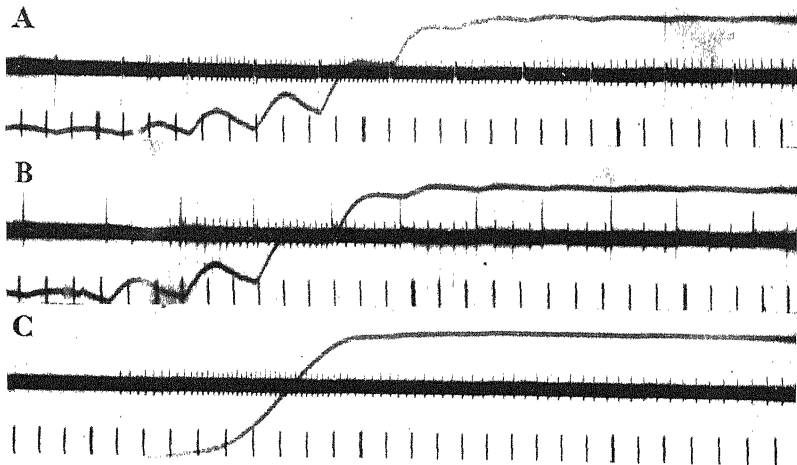


Fig. 13. Response from an A 2 ending in soleus. A, 110 p.c. stimulation muscle extended to approximately 300 g. tension. B, maximal stimulation, extension to 300 g. C, no stimulation extension to 300 g. approximately. Time marker 1/20 sec.

anatomical position of these receptors "in parallel" with the muscle fibres.

It has not yet been possible to identify the endings giving the A 1 response histologically as was done in the frog owing to the great number of nerve endings in the histological preparations that have been made. The position of an ending under observation can be roughly localized by pressing on the muscle with a glass rod and by local warming with an electrically heated pad while the muscle is subject to stretch. If this be done a region can be localized in many preparations where pressure produces the maximum response, and heating causes the greatest acceleration in the response to steady stretch. The method admittedly gives only very rough localization, but it is found that in general the receptors yielding the A 1 type response lie in the belly of the muscle, and in many cases they have appeared to lie near the top insertion of the muscle but never in the tendon.

In conclusion all the properties of the A 1 receptors certainly suggest that they are endings lying in the muscle spindles.

A 2 type of response.

The behaviour of these endings to passive stretch is practically identical with that of the A 1 type endings, though they often have a considerably higher threshold and the response rate rises more slowly with tension than does that of the A 1 endings; the chief difference in their behaviour lies in their response during tetanic contraction. Endings of this type form about 25 p.c. of those examined.

The difference between A 1 type of receptors and the A 2 type becomes clear when the strength of the stimulus evoking a motor response is varied. If the nerve be tetanized with submaximal shocks the cessation of response to stretch occurs as with the A 1 endings, but if the stimulus is made supramaximal the response is greatly accelerated during contraction. Behaviour of this sort has been observed in the frog [Matthews, 1931*b*] and ascribed there to contraction of the intrafusal muscle fibres occurring only with supramaximal stimulation; the same explanation fits the present observations very well.

The behaviour of an A 2 ending in peroneus longus is shown in Pl. I, Fig. 11 A and B. In B the stimulus is 15 p.c. submaximal, in A it is 10 p.c. supramaximal; thus an increase in the stimulus of a few per cent. completely transforms the end-organ's response, so that during contraction instead of the response stopping it is enormously accelerated. When the contraction is over the response rate in A falls below its original value;

presumably this is largely due to the adaptation brought about during the period of rapid response.

In Pl. II, Fig. 12, the behaviour of an A 2 ending in soleus is illustrated. The top records show the start of isometric tetanic contraction; in A the stimulus is just maximal, in B it is 20 p.c. supramaximal, in A the response to stretch ceases when the muscle contracts, in B it is accelerated. These phenomena are still more striking if during steady tetanic contraction the stimulus be increased from maximal to supramaximal. This is shown in Pl. II, Fig. 12 C; there is no visible increase in the strength of contraction with the increase of stimulus, but the end-organ formerly quiescent begins to respond. The conclusion of tetanic contraction with supramaximal stimulation is shown in Pl. II, Fig. 12 D. The continuity of the response during and after contraction (see also Pl. I, Fig. 11 A) leaves little doubt that the impulses occurring during contraction come from the same ending as those set up in response to passive stretch.

When the muscle is subjected to supramaximal stimulation at various initial lengths the response rate during contraction increases with the total tension in the muscle, and the response always occurs at a higher frequency than it does at the same external tension if the muscle is not contracting; with barely maximal stimulation the response frequency is far below that set up by the same passive tension. This is illustrated in Pl. II, Fig. 13. In A the nerve is tetanized supramaximally and the muscle extended until a tension of about 300 g. is reached, in B the stimulus is barely maximal, and in C the muscle is passively extended until the same tension is reached. The response rate in A is always higher, and that in B lower, than that in C.

Interpretation.

Thus the response of the end-organ is enormously dependent on small changes in the strength of stimulus applied to the nerve, though these do not produce any visible change in the total tension in the muscle. The most satisfactory interpretation of these observations is as follows. (a) That the A 2 ending lies in the muscle spindle; this is suggested by its behaviour during stretch and its "in parallel" behaviour during sub-maximal contraction. (b) That the threshold of the nerve fibres to the intrafusal fibres of the spindle is higher than that of the other motor fibres; this is in accord with the anatomical fact that these fibres are smaller [Ruffini, 1898], and Gasser and Erlanger [1925] have shown small fibres to be less excitable than large ones. When the threshold of the intrafusal motor nerve fibres is passed the nerve ending instead of

lying in a passive structure "in parallel" with those producing the tension, is stimulated by the contraction of the intrafusal fibres. This stimulation would seem to be mechanical rather than electrical or chemical, for firstly from A2 endings there is as a rule no response if the muscle contracts with its tendon unattached whatever the strength of stimulus may be, and as the chemical and electrical changes will still be occurring in the intrafusal fibres we must assume that they have little or no stimulating effect; secondly the rate of response during supramaximal tetanus is related to the external tension developed. So it appears that the acceleration of response during contraction may be due to the nerve ending lying in effect in series with the contracting intrafusal fibres.

In one preparation when the tendon was freed and the nerve stimulated supramaximally, each stimulation escape in the electrical record was followed by two or three impulses in close succession which were apparently travelling in the same fibre as that carrying stretch impulses. These did not appear if the muscle was under tension (when the response was like that of Pl. II, Fig. 13 B) and they did not occur when the stimulus was made submaximal. They might certainly have been due to excitation of the nerve ending by the action current of the intrafusal fibres, and their absence under tension may have been due to the decrease in excitability of the ending with adaptation to the tension. These impulses have only been observed in this one preparation (though all were examined in this way), and their absence in other preparations and in this one during stretch makes it unlikely that such impulses have any great reflex importance.

Response of the A2 endings during twitch.

In Fig. 14 A and B is shown the response of an A2 type ending in peroneus longus during isometric twitch; in A the stimulus is 85 p.c. maximal, in B it is 10 p.c. supramaximal; as in tetanic contraction, a slight increase in the stimulus causes the pause in the response to be replaced by a rapid discharge of impulses. The maximum rate of response occurs before the maximum tension is reached. In Fig. 14 C and D are shown records of the response of A2 endings in soleus when it contracts isometrically at two initial tensions, in both cases in response to supramaximal stimulation. At the lower tension there is a pause in the response, at the greater there is an acceleration during twitch. At the higher tension if the stimulus be submaximal the pause occurs as it does with A2 endings in peroneus longus at all tensions (see Fig. 14 A). Some light is thrown on this behaviour by the response during supramaximal tetanus at moderate tensions (Pl. II, Fig. 12 B); here the response is increased in rate during steady tetanic contraction, but only after the tension has risen to its new value, the first twitch in the tetanic series in Fig. 12 B leading to a cessation of response which is clearly similar to

that in Fig. 14 C. At greater initial tension the increased response is established at once during the first twitch of the tetanic series. This is shown in Pl. I, Fig. 11 C, and is similar to the behaviour during isolated twitch in Fig. 14 D.

Possible explanation.

It has been suggested from observations of the response during sudden stretch and release that the end-organ structure, particularly in soleus,

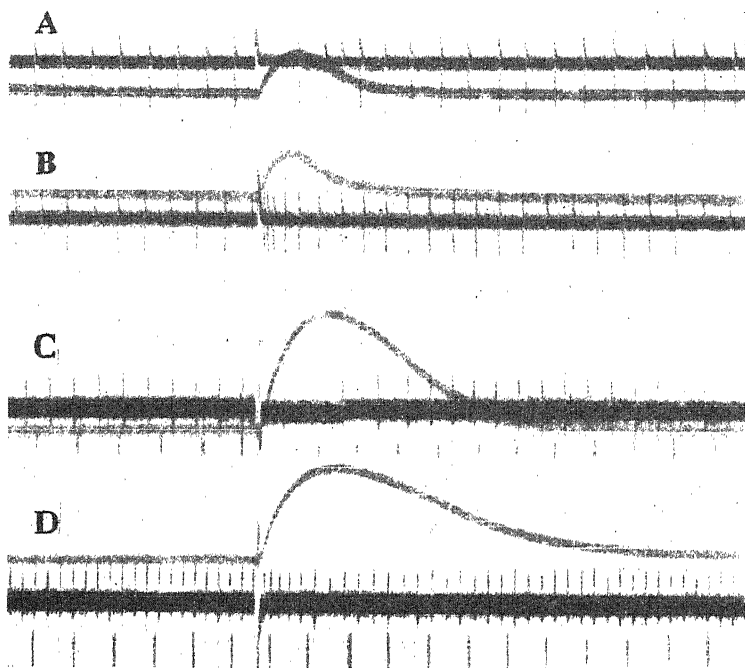


Fig. 14. Response from A 2 endings during twitch. A, B, peroneus longus, same ending, initial tension 40 g., stimulus 85 p.c. maximal in A, maximal in B. C, D, soleus 110 p.c. maximal stimulation, tensions 50 and 300 g. Time marker 1/20 sec.

may be more viscous than the rest of the muscle. That the viscous elastic properties of the intrafusal fibres may differ from those of the other muscle fibres is also suggested by their histological appearance; they are much finer and more coarsely cross-striated and contain very many nuclei. If this be so these results are very simply explained on the following lines. When the muscle contracts at moderate initial tension shortening will occur owing to the elastic yield of the tendon. Assuming

that with supramaximal stimulation the intrafusal fibres are contracting, we should expect a fall in the tension in the end-organ at the start of tetanization owing to the muscle as a whole shortening faster than the more viscous end-organ. Thus the response should at first cease or be reduced, and only rise to its new value after some time when the intrafusal fibres have shortened the same amount as the rest of the muscle. On the other hand at high tension the elongation of the tendon is negligible during twitch, so we might expect these effects of shortening to be absent.

Isotonic contraction.

If the muscle contracts isotonically the response ceases entirely if the stimulus be barely maximal; if the stimulus is supramaximal this still occurs as a rule, but in some preparations at all tensions, and all preparations at high (above 1000 g.) initial tensions, the response continues during isotonic tetanic contraction, though at a rate far lower than that set up before contraction. During isotonic twitch the pause is always absolute.

Response on relaxation.

From Pl. I, Fig. 11 A and B, it will be seen that during relaxation from isometric tetanus the response depends on the strength of the previous stimulus. On the view put forward above in Fig. 11 B the intrafusal fibres are passive, so that at the end of contraction the *status quo* at the nerve ending is restored and the response goes on again at its old level; on the other hand in Fig. 11 A and 12 D the intrafusal fibres are supposed to be actively contracting, and during tetanus their contraction will have caused them to shorten, so that on relaxation they will be re-extended. It will be seen that during relaxation the response is greater than it was before contraction, and this may perhaps be ascribed to this re-extension. Similar behaviour is seen on cessation of isotonic tetanus; if the stimulus has been barely maximal the response is far less during relaxation than if the stimulus has been supramaximal, though in both cases there has been little or no response during the contraction. This again may perhaps be ascribed to re-extension of passive or actively shortened intrafusal fibres.

Sharpness of change in response.

In all preparations of A 2 endings in peroneus longus and about two-thirds of those studied in soleus the change from silence to response during tetanization occurs very sharply, being produced by an increase of 1-2 p.c. in the stimulus, fine gradation of the stimulus failing to show

up any intermediate behaviour; moreover, the change often did not occur until the stimulus was supramaximal as judged by the height of contraction. But in about one-third of the soleus A 2 preparations the

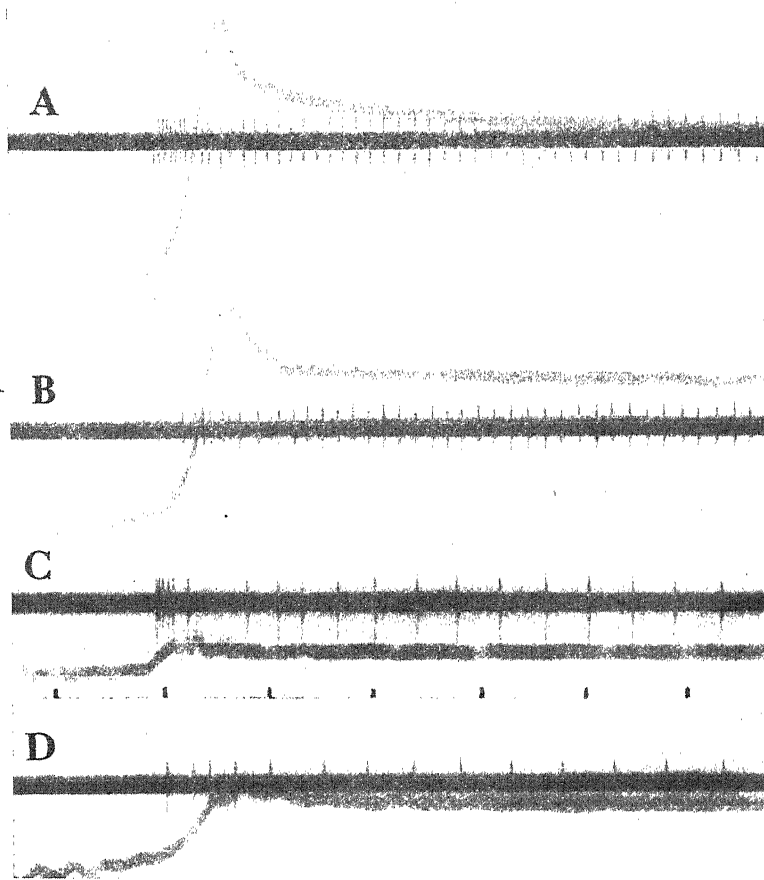


Fig. 15. Responses from A1 and B endings in peroneus longus during rapid stretch. A, A1 ending, extension 30 mm. per sec., final tension 35 g. B, B ending, extension 30 mm. per sec., final tension 140 g. C, A1 ending, extension 50 mm. per sec., final extension 10 g. D, B ending, extension 50 mm. per sec., final tension 60 g. Marks at foot of C indicate 1/10 sec. intervals.

change in response occurred before the contraction had become maximal and took place gradually as the stimulus was increased, with several intermediate stages between complete silence and full response. In these preparations the changes usually occurred as the stimulus was increased

from 70 to 90 p.c. maximal. This behaviour might be due to the intrafusal fibres in the spindle contracting severally, as it is known that in some cases several motor nerve fibres enter the spindle.

The B, C and other endings.

The B endings have been found in every muscle that has been examined. About 25 p.c. of the single receptors examined have been of this

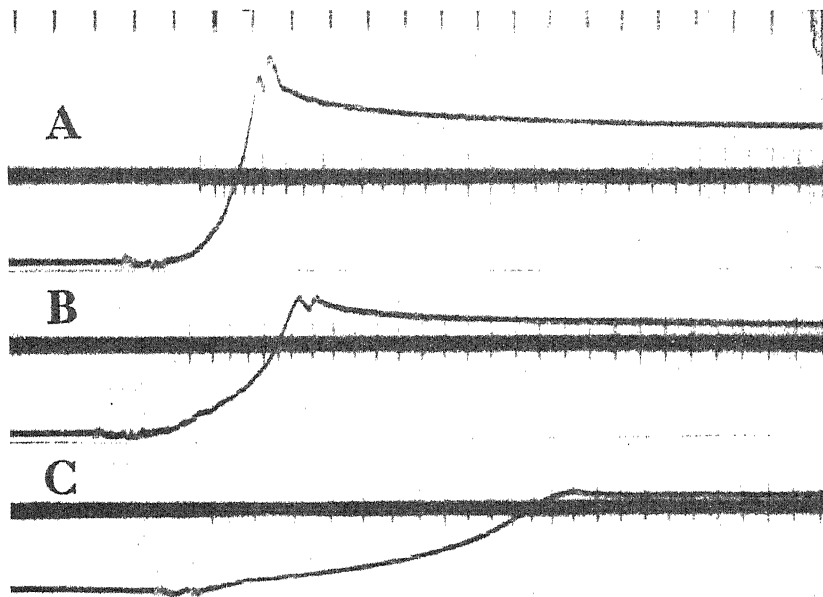


Fig. 16. Responses from a B ending in soleus. Stretch at three rates to a final tension of 260 g. Time marker 1/20 sec. at top.

type. They are very easily distinguished from the A endings by their behaviour during sudden stretch, for they do not give the rapid response characteristic of the A1 and A2 endings, and as a rule they have a far higher threshold; as a result they can be instantly distinguished by ear from the response in the loud speaker. The response during active contraction is also quite different from that of the A endings, for they respond as though they lay "in series" with the contracting elements whatever the strength of stimulus evoking contraction.

Response to stretch.

In Fig. 15 B and D are records from one of these endings in peroneus longus during rapid stretch, comparable records from A endings being shown in Fig. 15 A and C; Fig. 16 shows records from a B ending in soleus during stretch at various rates. Comparison with Fig. 5 reveals a striking difference in behaviour, for the response of the B ending seems to depend on the tension at any moment and to be only slightly affected by the rate

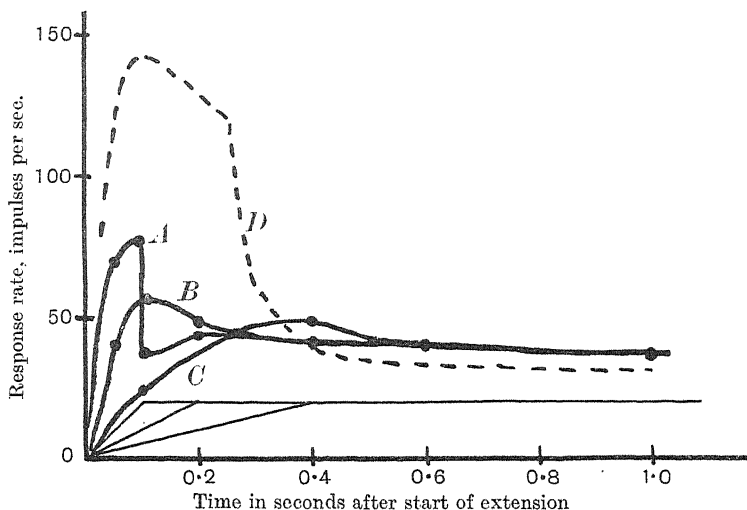


Fig. 17. A, B, C, graphs of response from a B ending in soleus during stretch to the same final tension at three rates indicated at the foot. D, graph of response of an A ending on stretch at a rate comparable to that giving curve B from the B ending (from Fig. 6).

of stretch, whereas that of the A ending is largely dependent on the rate of stretch. The response from a B ending in soleus stretched at various rates is shown graphically in Fig. 17, and one of the graphs of Fig. 6 from an A ending is redrawn to emphasize the very great difference in their behaviour. The behaviour of the B ending on quick release shown in Fig. 8 A also differs markedly from that of the A ending, in that the fall in the response rate is less marked, and the return of response follows more nearly the return of tension in the muscle after release.

Threshold.

The threshold of these endings in many preparations is far higher than that of A endings, which often respond in the absence of external tension

and always respond to a tension of 5-10 g. The B endings have never been found to give a response in the absence of external stretch, a tension of 20-200 g. usually being applied before the response begins, and in some preparations the threshold has been as high as 700 g. These endings can respond rapidly if the muscle is subjected to very high tensions, 5000 g.; but the rapid response suddenly falling to a much lower rate at the conclusion of the movement of extension cannot be evoked from them (the fall in Fig. 17 A correlated with the fall in total tension after rapid stretch). This strongly supports the view put forward above that this behaviour of the A endings is due rather to their anatomical position than to any common property of nerve endings.

Adaptation.

The adaptation curves of B endings are essentially similar to those of the A endings, but the phenomena noted above with A endings at high tension, that the response after falling slowly for a minute or more may suddenly stop or undergo transient acceleration followed by abrupt stop, are far more in evidence. If these phenomena be due to the effects of restriction of blood supply, this is to be expected, for the higher tensions necessary to stimulate these endings may produce a very great hindrance to the circulation.

Response during contraction.

The response during twitch at various initial tensions is shown in Fig. 18, and in Fig. 19 are records taken during maximal tetanic contraction, in A at low initial tension (soleus), in C at high initial tension (peroneus longus), and in B during steady contraction at various tensions evoked by maximal stimulation at various extensions. The records are similar to those in Fig. 19 B if the different tensions are produced by altering the stimulus at constant (considerable) stretch, though in a number of preparations as the stimulus is reduced the response drops out at a higher tension than it does if the extension is reduced with maximal stimulation. When the muscle contracts isotonicly the response is unchanged or may accelerate by a few p.c. It will thus be seen that these endings always behave as though they are in series with the contractile elements, the response depending simply on the total tension in the muscle whether this be produced by active contraction or passive stretch. This is emphasized if the response is recorded during slow passive extension to considerable tension and also when the muscle is tetanized and gradually extended; the rate of response at any tension is

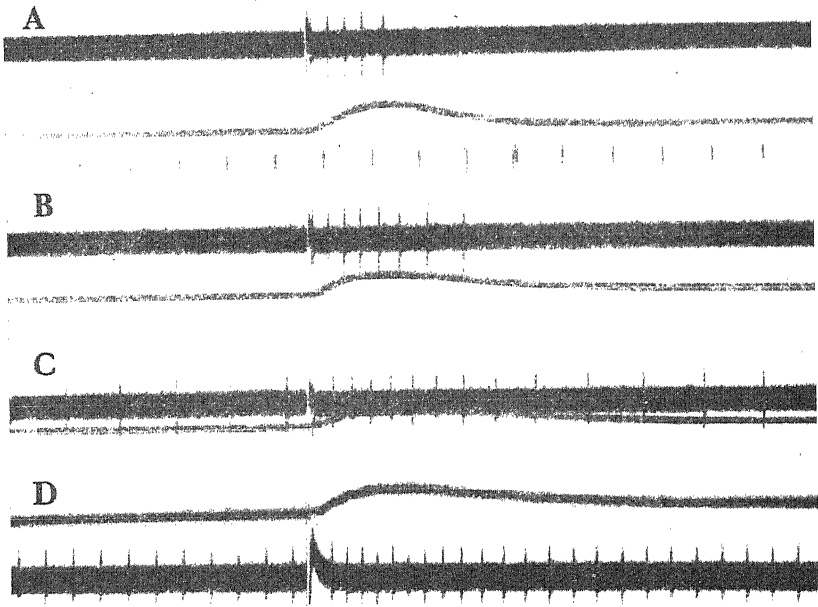


Fig. 18. Response from a B ending in soleus during maximal isometric twitch.
A, B, C, D, initial tensions of 40, 130, 240, 600 g.

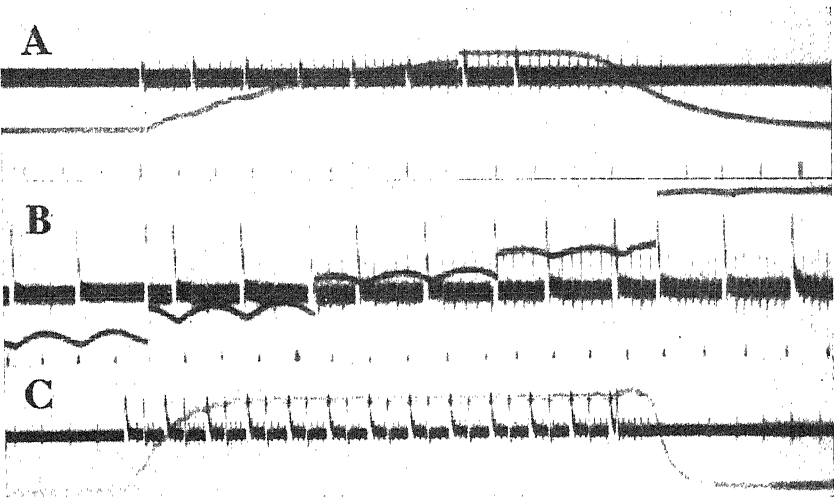


Fig. 19. Responses from B endings during maximal isometric tetanic contraction. A, soleus, initial tension 50 g. B, soleus, five records during steady tetanus, final tensions 50, 240, 470, 620, 950 g. C, peroneus longus, initial tension 80 g. Time marker 1/20 sec. at foot of A.

practically the same in the two cases, and in some preparations it is a little higher during active contraction, but there is never any great difference.

The above observations point strongly to the conclusion that the B endings lie in the attachments of the muscle and may be the tendon organs of Ruffini. Investigation of the position of the B endings by the warming and pressure methods described above accords with this view. In two cases B endings were clearly located in the tendon of the muscle (soleus); some appear to lie near the top insertion of the muscle, but the majority appear to lie in the muscle towards the tendon end. Ruffini's tendon organs are known to be present in these sites.

The C type endings.

These endings have only been met with in a few preparations. They do not appear to lie in the muscle but rather in the fascia associated with it, particularly that of peroneus longus; if the fascia be cleared away the C type of ending is never found, and it has never been found to occur in soleus when cleared from its neighbours. Response from these endings occurs during stretch when 5-20 impulses are set up at a rate depending on the speed of stretch, but there is no continued response to steady tension, often a brief discharge occurring if the muscle is suddenly released from considerable extension.

During active contraction the behaviour of C endings is very variable, and they never give any continued response during contraction; often a group of 2-5 impulses is set during the rise or fall of mechanical activity, sometimes during both. But from the small numbers in which they are present it is unlikely that their response can have any considerable central effect.

Other types of response.

In a few preparations endings have been met with that do not fall sharply into the above classification (some 4 p.c. of those examined); to stretch they behaved as A endings, but during contraction of the muscle their behaviour did not justify their classification as either A 1 or A 2, for though they always continued to respond during contraction the response slowed during contraction by variable amounts that had no clear relation to the tension on the muscle, no small alterations in the motor stimulation having any effect on the response. In two preparations slight acceleration occurred on contraction whatever the strength of stimulation, but its magnitude was not related to the tension on the muscle. These endings may perhaps have been A 1 or A 2 endings which behaved abnormally owing to some peculiarity of their attachment in the muscle.

No endings have ever been found that have responded to active contraction and not to passive stretch.

Time relations of impulses.

A full study has not yet been made of the time relations of the action currents from the A 1, A 2 and B muscle receptors, but some results of interest are already to hand. For these time relations to be compared it is imperative that impulses from each receptor be recorded successively from the same electrodes on the same stretch of nerve, otherwise many disturbing factors enter and make comparative measurements impossible [see Matthews, 1929*b*]. Thus to study this point it is necessary to make preparations from which the response of two receptors of different types can be recorded. With the nerve-section method of isolation this is solely a matter of trial and error; moreover, care must be taken to see that the two surviving sensory fibres are not damaged and are both conducting beyond the recording electrodes, otherwise the action potentials will be distorted. Such preparations have been made, and from high-speed records (taken with $0.25\mu\text{F}$ coupling condensers in the amplifier) the time relations of the action currents accompanying impulses from the A 1 endings appear to be slower than those from either A 2 or B endings.

In making single-ending preparations many two-ending preparations have been made and examined. Records taken with the $0.001\mu\text{F}$ condensers usually employed give some indication of the comparative time relations of the action potentials recorded monophasically, for the faster potential changes give records of greater amplitude than slower potential changes which are more reduced by distortion (in some experiments $0.0005\mu\text{F}$ condensers were used to this end). Thus the comparative heights of the deflections recorded give some indication of the rapidity of the process occurring, and providing that conduction of the fibres concerned up to the crushed region is verified by moving the electrodes (with monophasic recording decreased height of deflection might occur if the impulse is not conducted far beyond the first electrode) this indication should be quite reliable.

Records from preparations of two end-organs taken in this way are shown in Fig. 20. In I and II records from two B receptors are shown; in II the basic stretch is sub-threshold for both; in I it just causes one to respond. The amplitude of the deflection of the action potentials from both receptors is the same, and it may be concluded that they are transmitted by fibres of similar characteristics; this is always found to be the case with preparations of two similar endings. In III is a record from a preparation of one A 1 and one B receptor; the stretch is sub-threshold for the latter, but causes a vigorous response from the A 1 receptor, which ceases during

twitch and is replaced by the response from the B receptor (cf. Figs. 9 and 18), the much greater amplitude of the B impulses indicating that their action potentials are more rapid than those of the A 1 impulses. Similar records of impulses from A 1 and A 2 receptors show those from the latter to be travelling in fibres of faster characteristics than are the former. It thus seems very probable that the A 1 impulses travel in smaller fibres than do impulses from the A 2 or B receptors. No reliable

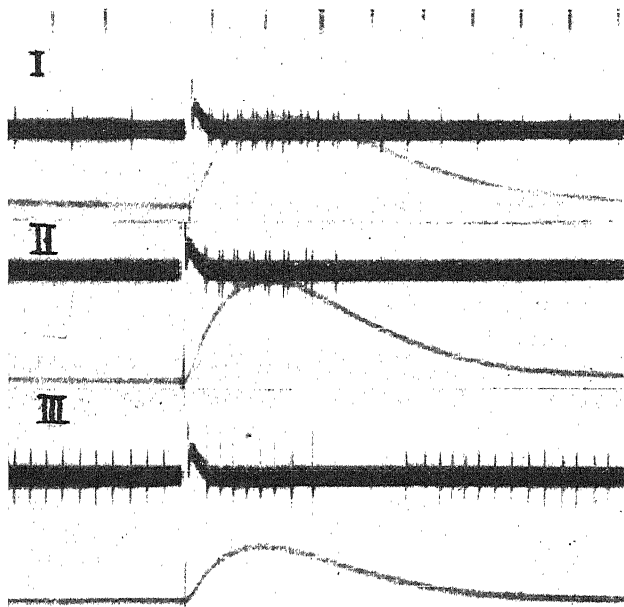


Fig. 20. Records of impulses from two receptors in the same preparation, during isometric twitch of soleus. I, II, two B type receptors, initial tensions 100 and 40 g. III, one A 1 and one B receptor, initial tension 40 g. Time marker 1/20 sec.

records have been made from A 2 + B preparations, so it is impossible to say whether fibres to these endings have identical characteristics, but the order of differences between A 1 and A 2 and A 1 and B impulses is similar, and so A 2 and B impulses cannot be very different.

Nature of the receptors.

We will here examine what correlation there is between the response of the stretch receptors and the known histological structures present in muscle. Evidence presented above (their "in series" behaviour, etc.) suggests that the B response comes from the tendon organs and the A 1

and A 2 response from endings (their "in parallel" behaviour, etc.) in the muscle spindle. Two histologically very different types of ending are present here, the flower-spray and annulo-spiral endings. For the reasons given below it is thought that A 1 response comes from the flower-spray endings, and the A 2 response from the annulo-spiral endings.

(a) Histological examination [*e.g.* Ruffini, 1898] shows the flower-spray endings to be supplied by medium sized nerve fibres, while the annulo-spiral and tendon organs are supplied by unusually large fibres. The time relations of the impulses from A 1 endings show them to be travelling in smaller fibres than those from A 2 or B receptors.

(b) The A response has been found in about twice as many single-ending preparations as the A 2 response. More fibres enter the spindle to supply flower-spray endings than to supply annulo-spiral endings.

(c) The A 2 endings appear to be stimulated mechanically by contraction of the intrafusal fibres, while the A 1 endings are not. Both A 1 and A 2 endings are stimulated by tension, which presumably acts by the distortion of their parts which it produces, for distortion produced by prodding a flaccid muscle also stimulates them; it is difficult to see how tension could stimulate except by the distortion it produces. The regions of the intrafusal fibres on which the flower-spray endings are found are apparently normal and clearly cross-striated, whereas under the annulo-spiral endings the fibres are poorly if at all cross-striated and are packed with nuclei. It seems probable that contractile activity is poorly developed in this nuclear region, and if this is so when the fibre contracts the nuclear region may be extended by the stronger contraction of the ends, and as a result the distortion of the annulo-spiral endings will be greatly increased while that of the flower-spray endings will be decreased. If this interpretation is correct the observed differences in their behaviour are clearly accounted for.

It thus appears very probable that the A 1 response is that of the flower-spray ending in the spindle, the A 2 response that of the annulo-spiral ending, and the B response that of the tendon organ. There are no observations that do not accord with this view.

Reflex effects.

An examination of the phenomena of the silent period makes it possible to suggest tentatively what the central effects from the various receptors may be.

In a previous paper [Matthews, 1931*b*] it was suggested from observations on the frog's spindles that the disappearance of stretch reflex

action currents from muscles which occurs during twitch evoked by either reflex or direct stimulation, might result from the cessation of response of the receptors normally evoking it. That this will play a part in the phenomenon seems inevitable, but observations [Cooper and Creed, 1926, 1928; Denny Brown, 1928] that not only do the stretch reflex action currents disappear from the muscle stimulated but also from associated muscles that have not contracted indicate either that during twitch impulses producing inhibition of the stretch reflex in other muscles are set up, or else that exciting impulses from one muscle contribute to the excitation of the centres of other muscles; there is no other evidence to support the latter alternative and the highly localized nature of the stretch reflex makes it improbable, and the former is supported by reflex behaviour.

There is thus reason to believe that during a twitch inhibitory impulses are set up; these impulses must be those coming from the B endings, for impulses only from these occur under all the conditions in which the silent period is in evidence. This is clear from the following considerations. The silent period is most in evidence at low basic tensions, and under these conditions the A 2 endings in soleus do not respond during twitch even if the intrafusal fibres contract (see Fig. 14 C). The A 1 endings never respond during twitch (Fig. 9), only the B endings respond during twitch at low tension (Fig. 18). So these alone can be held responsible for the inhibition. That B endings may produce inhibition agrees with the well-known cessation of stretch reflex that occurs when a muscle is stretched violently, and under these conditions the B receptors will produce a considerable response, but at lower tensions owing to their high threshold their response will be slight or absent.

The function of the A 1 endings appears to be excitation of the stretch reflex. The A 2 endings may also excite this reflex, but the very low threshold and numerical preponderance of the former suggest that they will be the dominating influence in this. If this is so, the silent period appears to be due both to the absence of excitatory impulses from the A 1 (and perhaps A 2 endings) and to the inhibitory impulses set up by the B endings. Fig. 20 III illustrates this point very clearly. During twitch, the supposed excitatory impulses from the A 1 ending cease, and the supposed inhibitory impulses are set up by the B ending.

If the time relations of impulses play a part in determining their central effect, there are differences between those of the exciting A 1 impulses and inhibitory B impulses amply sufficient to account for their very different central effects. But the time relations of A 2 and B

impulses are very similar, so if these determine the inhibitory effect of B impulses, the A 2 impulses should also produce inhibition. This may indeed be the case, but there is no possibility of a decision on this point until we know under what conditions of central excitation the intrafusal fibre contracts, for the response of the A 2 endings is entirely dependent on this. If the A 2 impulses are inhibitory we must imagine that in a stretch reflex a balance is struck between excitation and inhibition in favour of the former, owing to the greater excitability and number of A 1 receptors. During twitch this excitation and inhibition will both cease and be replaced by the B ending inhibition. What will happen in tetanic contraction depends entirely on the intrafusal fibres; on the whole a balance in favour of inhibition might be anticipated, so that a contracting muscle would always tend to inhibit its own contraction! It is clear that evidence of the central exciting or inhibiting effect of A 2 endings must come rather from observations on reflex effects. From the present work A 2 impulses might be either inhibiting or excitatory; the evidence above is slightly in favour of their being inhibitory, and existing reflex evidence also suggests this conclusion.

PART III. FACTORS AFFECTING THE RESPONSE OF STRETCH RECEPTORS.

Effects of circulatory arrest.

The effects of arresting the circulation to the muscle on the response of end-organs lying in it are extraordinarily interesting, for not only do they shed some light on the mechanism of the end-organ, but they are also of interest in connection with the intense muscular pain that occurs in limbs with occluded circulation that has recently been studied in detail by Lewis, Pickering and Rothschild [1931]. The effects observed here depend to some extent on the state of the animal, as there are considerable differences in these effects in spinal, decerebrate, and anæsthetized animals. The effects in decerebrate animals will be considered in greatest detail, as these are in a state most nearly approximating to that of the living animal.

Decerebrate animals.

In decerebrate animals it has been found a matter of some difficulty to stop the circulation by clamping the arteries, as owing to the high blood-pressure (as compared to spinal animals) collateral circulation which is difficult to locate is often quite effective in maintaining the blood supply. The methods used to arrest the circulation have been, clamping the femoral artery where it leaves the abdominal cavity, clamping the arteries

as close to the muscle as is practicable, and tying a cord tightly round the thigh.

Many of the A 1 and a few A 2 endings respond with the pulse, and at each heart beat one or two impulses are set up when the muscle is slack. This response vanishes instantly on clamping the arteries, but no other immediate change occurs in the behaviour of the nerve ending; it thus appears that the blood-pressure does not have any appreciable mechanical effect on the end-organ.

If the circulation is arrested, and precautions are taken to prevent cooling of the limb, the response to stretch from a single nerve ending of any of the types described at first increases up to 20 p.c. or less, then it becomes gradually smaller until after 5–20 min. there is only a brief response when the muscle is extended. During this period of lowered excitability the ending will still set up a continued response when a very large load is applied to the tendon. After a further lapse of some minutes the response returns and increases, so that a given stretch sets up a greater and greater discharge; after half an hour of hyperexcitability the response to stretch gradually becomes less and less but may be still present $1\frac{1}{2}$ hours after the occlusion of circulation. A resting discharge in the absence of stretch also appears as the hyperexcitability develops, and it retains the characteristic of the normal resting discharge that after stretch it stops or is at least reduced in frequency. In a few preparations this discharge has risen to a rate of about 50 per sec. After this occurs it rises rapidly to a rate of 300–400 per sec., and after it rises above 300 per sec., with this high rate of discharge, stretching the muscle causes only a slight acceleration, and there is only a slight slowing on release. The discharge continues at 300–500 per sec. for 1–4 min. and then falls rapidly, and after a few seconds' irregularity ceases abruptly. No further response can be evoked by stretch. If the circulation is now released after 2–10 sec. the rapid response suddenly restarts and falls to extinction in 1 or 2 sec.; after a few minutes' rest the end-organ is once more in its original condition and the whole sequence of events may be repeated.

Occlusion and motor stimulation.

The sequence of events described above occurs far more rapidly and the rapid spontaneous response occurs in every preparation if the muscle is stimulated *via* the nerve after occlusion of the circulation; 1 min. tetanization is often sufficient to bring on the spontaneous rapid response. If this tetanization is applied in 5 sec. bursts, at intervals of 10 sec., and after each the state of the end-organ be examined by recording its

response to stretch, it is found that the excitability of the ending changes in the same way as it does after occlusion without stimulation but far more rapidly. The response under these conditions is shown graphically in Fig. 21; the full line shows response to steady tension, the dotted curve shows the result of repeating the experiment with no tension on the tendon. When the response reaches a frequency of about 100 per sec., even without further stimulation, its rate rises rapidly to 350–500 per sec. and is

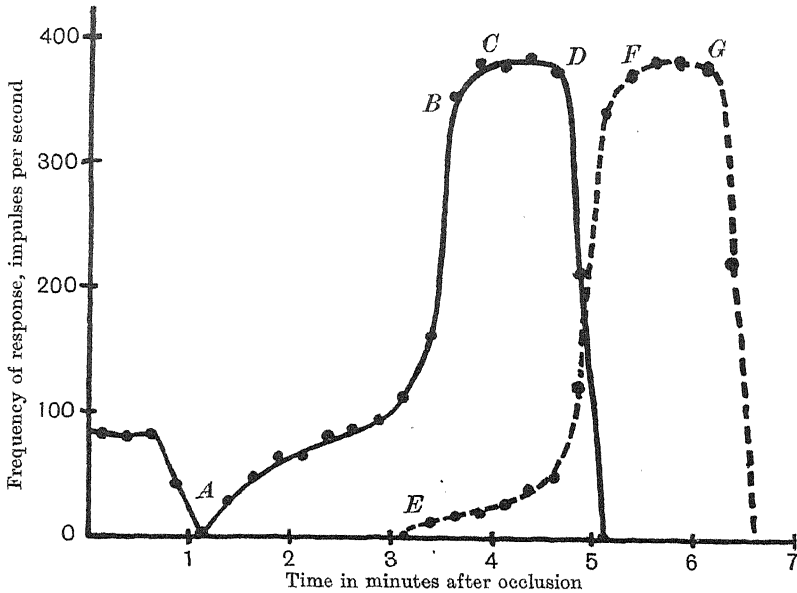


Fig. 21. Graph of response from an A1 ending after occlusion of the circulation. The nerve is tetanized for 5 sec. at 20 per sec. every 15 sec. The full line shows the response when 50 g. tension is maintained in the tendon, the dotted line that occurring if the muscle slack.

maintained for 1–4 min. before it fails; but with preparations in which a number of endings are under observation, after a burst of stimulation that initiates the spontaneous discharge from some of the endings present, though the response from these rises to a maximum and after a few minutes fails, new endings are recruited to the spontaneous discharge slowly, whereas further stimulation will initiate the rapid response from a large number of endings not previously responding spontaneously. When the response of the whole nerve is observed, after each burst of stimulation a number of endings “explode,” but the total response does not increase further until after a further burst of stimulation and may even die down after a few minutes. The first nerve endings to “explode”

do so before the mechanical response has fallen noticeably (from fatigue), the last often do not "explode" until the contraction of the muscle has become feeble.

These observations suggest that some factor accumulates on stimulation, which causes the excitability to rise and initiates the spontaneous response, but that the course of this response once started is determined by the activity itself rather than by further changes in the excitability brought about by the initiating factor; for after stimulation the response of an ending which has begun to respond spontaneously rises rapidly, though the exciting factor does not seem to be increasing sufficiently to recruit other endings immediately.

That this may be so is supported by the following observations. If an ending behaving like that in Fig. 21 have the stretch stimulus removed at any point B-C the response falls immediately but then continues to rise and the rapid response occurs earlier and thus after less stimulation than it does if the stimulus, and therefore response, be absent during the period AB; so the attainment of the spontaneous response state is accelerated by the activity during this period. Secondly, if the muscle is unloaded at C the rapid response continues and its termination is unaltered so that it must result from the activity A-C, for if the muscle be unloaded at A the response follows the course EFG, therefore the failure at D appears to result from the activity A-C. Thus though the preliminary changes in the excitability are due to the lack of circulation, the course of the final rise and extinction is determined rather by the activity of the nerve ending which appears to initiate a self-destructive activity.

If the circulation is released immediately after the failure of an ending the rapid response restarts and falls quickly to zero in 1 or 2 sec.; but if after failure further stimulation is applied, this does not occur on release, but some minutes later the ending is found to have returned to its normal resting state.

If the circulation is released while an ending is responding rapidly, the response begins to fall rapidly 1-5 sec. later, and ceases after 1-3 sec., even if bursts of stimulation be still applied to the nerve.

Constancy of behaviour.

Although there are these variations in the attainment of the spontaneous discharge in different preparations, in any single-ending preparation the changes in the response after successive clampings of the artery agree very closely. When the time taken to reach the spontaneous response has once been observed, the moment when the response will begin and reach its height can be predicted within 10 sec. for future clampings, for if the muscle is allowed 10 min. rest after each series of observations the whole sequence of events is exactly repeated.

With the preparation in Fig. 21 the spontaneous discharge was rising rapidly 5 min. after occlusion with stimulation every 10 sec. for 5 sec.;

when the occlusion was repeated and continuous stimulation applied for 100 sec., on cessation the spontaneous response was again found to be rising rapidly; thus the incidence of the spontaneous response depends much more on the total stimulation that has been applied than on the time that has elapsed since circulatory arrest. This is found to be the case however the stimulation is spread out, though with prolonged occlusion the spontaneous response occurs with less stimulation than in brief occlusion, so that the time factor does also come in; this is also shown by the fact that in a few preparations the response has been obtained with no stimulation but prolonged occlusion.

When the behaviour of several identified endings in a muscle is examined it is found that the A 1 and A 2 endings give the spontaneous discharge sooner after clamping with stimulation than do the B type endings. Only three observations have been made on this; but they agree with the observation above that these endings have a high threshold to stretch and are presumably less excitable, and if as seems likely they are tendon endings they may well be less in the path of chemical changes occurring in the muscle than the endings in the spindles.

All stretch receptors behave in this way.

All the stretch receptors present in a muscle appear to go through these changes in excitability after motor stimulation. The response of the whole nerve can best be followed by ear with the loud speaker. When each ending producing a rapid response gives rise to a siren-like wail from the loud speaker, six or more spontaneous responses can sometimes be heard simultaneously; the maximum frequencies set up by the different receptors are of the same order but not identical, so that a number of notes are heard (from aural observations it is thought that these notes may fall into two groups, one of rather higher pitch than the other). When a further ending starts its spontaneous discharge a rising tone is heard very like that of a rotary factory siren starting up. By counting the number of these rising tones after each burst of motor stimulation it is possible to estimate the total number of endings in a muscle behaving in this way; in soleus about 40 "exploding" nerve endings can be counted after stimulation. After some minutes' stimulation all the endings present appear to have undergone "explosion," for there is no continued response even to large loads, and a few impulses are set up during extension only. A peculiar feature that has been noted in several preparations is that if during the spontaneous rapid response the muscle is violently (2000 g.) stretched the response ceases and recommences on release.

Spinal animals.

In these the spontaneous discharge is brought on with extreme ease, even without stimulation, often only a minute after occlusion if the nerve is not stimulated or after 10–40 sec. when it is. Moreover the spontaneous response appears sooner and sooner after clamping as time elapses after decapitation, and after 4–5 hours the endings seem to be in a hyper-excitable state even with uninterrupted circulation. The ventilation was not measured and was probably excessive, but even the ventilation necessary to ward off convulsions appeared much greater than that of a decerebrate animal. For these reasons spinal animals were not used in working out the details of end-organ behaviour described earlier in this paper. If the respiration pump is stopped spontaneous discharges begin with the onset of convulsions.

Anæsthetized animals.

With animals under urethane the spontaneous discharge has never been obtained. When the circulation is clamped, after brief hyper-excitability the endings become very unexcitable, and later the excitability rises above normal but never so high that the spontaneous response occurs even if the nerve is stimulated until the muscle has ceased to contract. Similarly if ether, chloroform or urethane is administered to a spinal or decerebrate animal, the spontaneous response previously occurring on arrest of the circulation cannot be made to take place. In several preparations during induction of chloroform and other anæsthesia a rise of excitability was found, indicated by a greater response to a constant stretch.

Discussion.

As a result of circulatory arrest the nerve ending undergoes great changes in its excitability, and this seems to be closely connected with the metabolic activities of the surrounding muscle, though as yet it is impossible to suggest what factor is responsible for these changes. The hyperexcitability found in spinal animals probably results from the excessive ventilation disturbing the balance of the blood in regard to this factor. Anæsthetics appear to reduce the susceptibility of the nerve endings to it, so that the spontaneous rapid discharge is impossible, but they only slightly affect the response to stretch, at first increasing it, later in deep anæsthesia it is a little reduced, but it is difficult to determine the origin of this effect since anæsthesia is also accompanied by changed

ventilation which probably in itself alters the end-organ response; in the extreme case of this, clamping the trachea of a decerebrate animal leads to spontaneous response in the endings in its muscles soon after the onset of convulsions; it does not seem unlikely that the convulsions may be intensified by the reflex effect of excessive impulses from hyperexcitable endings in the muscles.

The rapid discharges which occur in the absence of an external stimulus bear a remarkable resemblance to those recorded by Adrian [1930] from damaged nerve fibres, and suggest that as a result of muscular activity and lack of circulation, the nerve ending breaks down and allows its nerve fibre to behave as though it were cut. One of the most notable features of this phenomenon is that the whole process is reversible, and that recovery from such catastrophic changes in the nerve ending can occur quite rapidly.

The spontaneous discharge seems to originate in the end-organ or in some part of the nerve fibre very closely associated with it, for the rise of excitability as judged by the response to stretch and by the spontaneous discharge run parallel; the spontaneous discharge is evidently originating from the same region as the normal resting discharge and stretch response, for its frequency is reduced after stretching. When the spontaneous discharge reaches its height each impulse is set up very soon after the end of the absolute refractory period of its predecessor, and only then is the rate unaffected by stretch.

Since there is no reason to suppose that the end-organ differs essentially from other excitable structures, where it is generally supposed a propagated disturbance is due to a breakdown of a polarized surface, we may assume that tension on the end-organ stimulates it by deforming the surface membrane sufficiently to produce complete instability. The breakdown is followed by a refractory phase and gradual return of the membrane to its normal polarized condition, but the continued action of the stimulus causes a renewed breakdown as soon as recovery has advanced far enough for the stimulus to become effective. In a previous paper from observations on single receptors it was suggested that adaptation might be due to a depletion of some substance necessary for repolarization inside the membrane or its accumulation outside, leading to a slowing of the recovery process and so of the rate at which impulses are discharged. The observations in this paper are in agreement with the above view of the end-organ mechanism. Moreover, it is supported by the continuous change which occurs in the end-organ response when the circulation is arrested, from that proper to a nerve ending to that set up by a damaged

nerve fibre. We may suppose that the increasing excitability is due to some factor which accumulates on stimulation of the muscle, making the membrane more permeable and unstable until finally it becomes completely permeable and is virtually a cut fibre; the adjacent regions of the fibre then respond at a rate determined by their refractory period and rate of recovery, as those of a cut fibre are known to do. The instability resulting from muscular activity appears at first sight to be comparable to that produced by stretch. But some differences are evident, for while the instability and activity resulting from stretch are accompanied by slowing of the recovery process evidenced by adaptation and a slower response, the activity resulting from muscular contraction appears itself to increase the response rate. This might be due to the instability increasing so rapidly under these conditions that the effects of slowed recovery are swamped. The termination of the spontaneous response may perhaps be due to a total failure of other parts of the fibre in the end-organ occurring owing to the great activity. On readmission of circulation progressive repolarization appears to occur very rapidly.

If the nerve-ending membrane behaves like that of a nerve fibre, the above changes accord with effects observed in nerve fibres. Recent work on the after potential and retention of negativity in nerve [Amberson and Downing, 1929; Gasser and Erlanger, 1930; Amberson, Parpart and Sanders, 1931; Furusawa, 1929] has shown: (a) That reduced polarization of the nerve membrane is associated with hyperexcitability. Gasser and Erlanger found that the phase of supernormal excitability in recovery was present under the conditions which lead to the appearance of after potential, and that the two phenomena seemed to be inseparable. (b) That the passage of impulses reduces the membrane polarization, and can even depolarize it completely in crustacean nerve. (c) That such depolarization occurs readily in the absence of oxygen. (d) That oxygen appears to be necessary for maintenance of the polarization on which transmission of impulses depends, rather than for the actual transmission. Thus these phenomena observed in nerve endings can be accounted for if we assume that the polarized membrane of a nerve ending has all the properties ascribed to that of the fibre in greater or less degree. To explain the differences in adaptation of a nerve fibre and ending several assumptions are necessary, but some of these differences [see Matthews, 1931 b] may well be connected with the fact that whereas in nerve fibres a symmetrical stretch of polarized membrane is observed, in nerve endings the fibre comes to an end and its modified behaviour may be due in part to its asymmetry, rather than to any fundamental differences in the properties

urized membrane. There is no experimental evidence that at present in ascribing to the nerve-ending membrane properties shared by its nerve fibre.

Central effects of the above phenomena.

It does not seem at all unlikely that great changes in the excitability of muscle receptors may occur during severe muscular work (similar to the effect of turning on activity with occluded circulation though lesser in degree) and this may in part contribute to the central fatigue resulting from it. The central effects of occlusion of the circulation to muscles have been studied in detail by Lewis, Pickering and Rothschild [1931], and it has been shown that in man intense muscular pain occurs if a muscle is allowed to contract after circulatory arrest. The conditions that lead to this are exactly similar to those that lead to the spontaneous rapid discharge of impulses from the muscle receptors studied here. The most striking similarities of the phenomena are as follows: (1) Both the pain and the spontaneous response appear when a definite amount of muscular activity has taken place after occlusion. (2) On release of the circulation the pain diminishes very rapidly, so does the spontaneous discharge. (3) If the muscle has been contracting just before the circulation is arrested, the pain is less than when it is elicited on with less activity than is necessary to elicit it if such activity has not taken place; this is also true of the spontaneous discharge. (4) On cessation of muscular activity the pain stops increasing in intensity, but it rises with further activity; the total spontaneous discharge from all the receptors in a muscle also remains steady or falls a little when activity ceases and rises again on further activity. The two curves appear to be exactly parallel, and we must conclude either that the same factor resulting from activity of the muscle affects both the pain and pain endings in the same sort of way, or else that the responses from stretch receptors are interpreted as pain. Lewis has ascribed the pain to an accumulation of "factor P " which results from activity and stimulates pain nerve endings in the muscle, but it is probable that small fibres associated with the blood vessels are concerned; occlusion and activity may affect the excitability of these in the same way. It is probable, and they may indeed be responsible for the pain. In fibres not carrying stretch impulses have not been detected as studied here, but they may well be present and pass unnoticed owing to the smallness of the potentials accompanying the small fibres. However, it is very hard to believe that the sensory discharge from the stretch nerve endings produces no

central effect, and it does not seem improbable that it might itself be responsible for the pain. It is most unlikely that such fundamental changes in the stretch receptors occur in the cat, and not in man, for these receptors are very similar; presumably the rapid response from the stretch receptors occurs in man, but if so it does not produce any obvious reflex contraction of the muscle, nor prevent its voluntary contraction; this might be due to a balance of excitatory and inhibitory effects, but this does not seem probable; thus the rapid discharge does not appear to be able to evoke reflex effects of the kind that are probably evoked by discharges at lower frequencies. If the discharges discussed here are responsible for the pain, then the same nerve fibres must convey impulses that have quite different central effects from those normally evoked by impulses occurring at lower frequencies in the same fibres; that this might occur accords with other observations on the central effects of sensory impulses discussed below.

There is a good deal of evidence that the size of sensory nerve fibres is related to the type of end-organs in which they end, and so to the sensations which they arouse [see *e.g.* Gasser and Erlanger, 1929]. In a previous paper [Matthews, 1929*b*] it was shown that cutaneous and muscular impulses in the frog were distinguishable from the time relations of their action currents. It is not unlikely that the time relations of impulses arriving at the C.N.S. in part determine their effect there and perhaps determine the paths that are open to them. For if the dendrites of second-order neurones vary in their excitation time characteristics, incoming impulses of particular time relations may pick out and stimulate particular second-order fibres.

If this is indeed the case we might anticipate that the rapid discharges described above would have central effects different from those of the normal slower discharges in the same fibres that subserve muscle sense, for during the rapid discharge each impulse is set up early in the relative refractory phase by its predecessor, and as a result the time relations of its action current will be considerably slowed [cf. Gasser and Erlanger, 1925; Matthews, 1931*a*]. Thus the electric responses of the impulses in the rapid response will be much slower than those of the impulses normally set up by stretch and will become more like those of the small fibres generally considered to transmit pain; this might well lead to their exciting different regions in the C.N.S. and interpretation as pain instead of as muscle sense. In this connection it is interesting that stimulation of a muscular nerve through the skin at 500 per sec. is extremely painful, and the pain has the character of that resulting from activity of muscles

with occluded circulation. Stimulation at 150 per sec., though unpleasant, is not very painful. These results are complicated by the contraction of the muscles resulting from stimulation, but certainly agree with the point of view put forward here that high frequency may in this case lead to impulses to be interpreted as pain.

However high frequency discharges in all nerve fibres cannot evoke pain, for Adrian, Cattell and Hoagland [1931] have shown that rapid intermittent stimulation of the skin does not elicit any sign of pain from an intact frog, though impulses at a high frequency are certainly set up under these conditions.

The effects of antidromic impulses on the stretch response.

A method which has been used to analyse the mechanism of diverse rhythmic structures is to observe how the spontaneous rhythm is affected by additional activity induced by electrical stimulation. This method has been used in the present work to examine the rhythm of the nerve ending. The effects of antidromic impulses on the stretch response have been studied in a number of preparations. The animal must be curarized to abolish the contraction of the muscles when the nerve is stimulated. To this end 1–2 c.c. of 1 p.c. curare in saline were injected intravenously, and 1–2 c.c. subcutaneously, for unless the subcutaneous injection was given the curarization was found to pass off slowly after $\frac{1}{2}$ –1 hour; artificial respiration was given. The curare has not been found to have any marked effect on the response to stretch.

Results.

It was found in the frog [Matthews, 1931*b*] that the end-organ response was “reset” by antidromic impulses, but these caused very little, if any, delay in the setting up of the next impulse by the rhythmic mechanism even if the antidromic impulse occurred early in the recovery cycle of the ending. But in the cat a considerable delay follows an antidromic impulse early in the cycle; the similarity of behaviour of the cat’s and frog’s receptors in other respects makes this difference the more surprising; no satisfactory explanation of this dissimilarity is at present apparent.

In the cat if a descending impulse is set up in the nerve, unless it meets an ascending impulse it apparently reaches the region of the end-organ that determines the rhythm, for this is “reset” and follows on from the descending impulse. This is illustrated in Fig. 22. Even if the descending impulse is set up immediately (0.002–0.003 sec.) after the arrival of an

ascending impulse the rhythm is reset, so this impulse must have reached the region determining the rhythm and activated it; thus under these conditions the absolute refractory period of this region cannot be of a very different order from that of the nerve fibre.

The length of the interval following a descending impulse depends on the position of this impulse in the rhythmic cycle of the end-organ, this may be seen in Fig. 22. In the top record where the descending impulse follows soon after an ascending one the succeeding interval is considerably

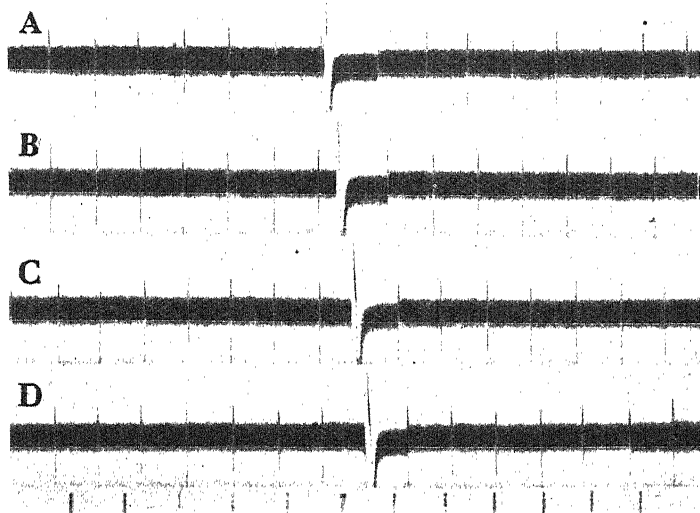


Fig. 22. Records showing the effect of antidromic impulses on the rhythmic discharge of an A1 ending. In A-D this impulse occurs at different times in the rhythmic cycle of the nerve ending. Time marker 1/20 sec. at foot.

longer than in the bottom record, where the descending impulse occurs late in the rhythmic cycle. The interval following the descending impulse increases, as this impulse occurs earlier in the cycle. This is shown graphically in Fig. 23 at two rates of rhythmic response from the same nerve ending.

The pause following the descending impulse is made up of: (a) The time taken by this impulse to reach the rhythmic structure. (b) The time taken by the latter to recover sufficient excitability to set up an impulse in response to stretch. (c) The time this impulse takes to reach the recording electrodes. The conduction time (a)-(c) will be of the order 1-2 thousandths of a second and will be the same at any rhythmic rate.

(b), on the other hand, depends on the position of the descending impulse in the cycle and on the external stimulus (stretch).

Rate of response.

The graphs of Fig. 23 show the intervals measured from the records expressed as percentages of the mean rhythmic interval. Clearly to obtain the times of recovery of the rhythmic structure we must from these subtract the time occupied in conduction; if this is taken as 0.0015 sec. it forms 1 p.c. of the interval in A (rate 7 per sec.) and 11 p.c. of that in B (rate 70 per sec.). If we subtract 11 p.c. from the intervals in A and 1 p.c. from those in B the results will represent more nearly the recovery time of the rhythmic structure. The graph B has been lifted

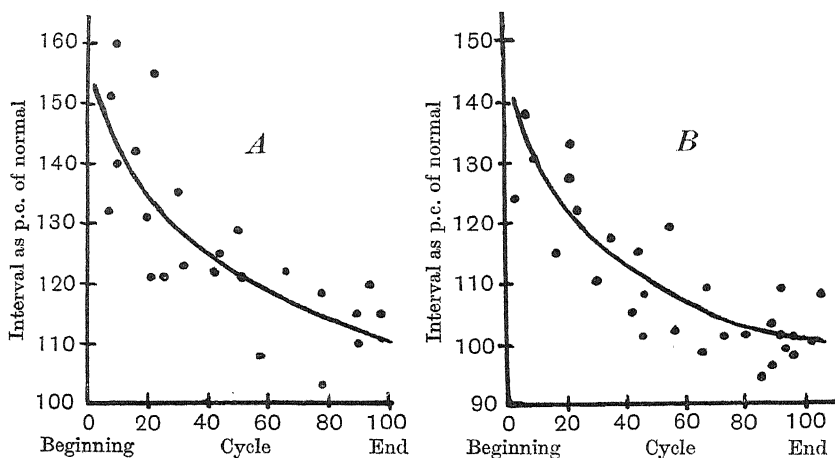


Fig. 23. Graphs of the interval following an antidromic impulse (expressed as a percentage of the interval of the rhythmic response) plotted against the position of that impulse in rhythmic cycle of the nerve ending. A, mean rate 70 per sec. B, mean rate 7.1 per sec.

10 p.c. relative to A for this reason, and it will be seen that the two graphs read on the ordinate of A are very similar. A similar correction for conduction time might be applied to the abscissa, but this has been omitted for simplicity. It is clearly impossible to activate the ending by a descending impulse until at least 0.0015 sec. has elapsed after its own activity; at a rate of 70 per sec. 10 p.c. of the cycle will thus elapse, and cannot be examined by this method.

It is clear that the recovery interval following a descending impulse (expressed as a percentage of the normal interval of the rhythm) varies with its position in the rhythmic cycle in roughly the same way at widely different rates of response, though at a slow rate the delay following the early descending impulse has a far greater absolute value.

It is found from a large number of measurements that after the prolonged interval following the early descending impulse the next interval is always normal.

Several antidromic impulses.

If a number of descending impulses are set up at a rate only slightly greater than those previously set up by the ending, the interval following the last before an ascending impulse appears is practically normal (quite normal if conduction time be allowed for). However, if the frequency of descending impulses is much greater than this ($\times 5$) there is an interval of up to 170 p.c. of the normal before a stretch impulse appears; this interval is longer after 10 than 5 descending impulses but no greater for 20 or 50 than after 10; and the interval following this one is normal.

Thus after a number of descending impulses the interval following the last increases with the number of impulses up to about 10, and increases with the frequency of the descending impulses; the exact relationship of these quantities has not yet been fully worked out.

Theoretical treatment.

On the view set out above we imagine that after an impulse is set up the excitability of the receptor is lowered and returns to the level at which a further impulse is set up by the stimulus at a certain rate, the rate of this return of excitability itself determines the rate of response. It would appear that as the stimulus increases, the rate of return of excitability to this level also increases, for the response frequency becomes greater. This may be due to either or both of the following alternatives: (a) that the process of recovery of excitability is occurring more rapidly; (b) that the degree of recovery necessary for response decreases with increase of the stimulus.

A descending impulse early in recovery appears to be able either to lower the excitability of the ending more than other impulses of the series do, or else to delay the recovery process, it is impossible to determine which; but if activity in the ending is followed by an absolute refractory period, we should expect any impulse to lower the excitability to this rock-bottom level, and the prolonged interval might be attributed to slowed recovery. The similarity of Fig. 23 A and B suggests that the slowing produced continues throughout the whole recovery cycle, otherwise at the slower rate the delay caused by the descending impulses would not be the same fraction of the normal recovery time. When several descending impulses are set up at about the rate of the ending's own response, unless a descending impulse travelling over the polarized

surface of the ending leaves it in a state different from that left by an ascending impulse we should not expect there to be a prolonged interval after the last, for conditions of activity have not differed in any way from those of the ending's own response. If the impulses descend at a rate much greater than that of the ending's own response the interval after the last is prolonged, so that recovery to the response level is taking much longer than after any impulse of the ascending series. It is very surprising that the factors responsible for this do not make their presence felt beyond the first ascending impulse, but as the second appears after the normal interval they clearly do not, and the termination of the first prolonged interval by an impulse indicates that excitability has returned to a definite value—that at which response occurs to the stretch. Apparently after this the recovery rate is absolutely normal. It thus appears doubtful whether the slowing of recovery observed here can be ascribed to the same causes that lead to the progressive slowing of the response during activity ascribed to adaptation, which develops and disappears more slowly.

The transient lowering of excitability at the ending appears to be much the same whether caused by an ascending or descending impulse towards the end of the recovery period. In a previous paper [Matthews, 1931*b*] effects were described in frog's receptors, which suggested the hypothesis that descending impulses could reduce the adaptation of a nerve ending. In the present work no clear indication of this has been found, but in some preparations after rapid stimulation a consistent acceleration of 1–2 p.c. was found to follow the prolonged first interval after cessation of stimulation, and to last for several seconds; this acceleration is of the same order as the random irregularities that occur in the rhythm, but its consistent appearance in the preparations in which it was found suggests that it may be present here, but be far less marked than in the frog. As with the frog in many preparations no sign of it was to be found. In the cat's receptors the rhythm falls far more slowly with activity than it does in the frog, the smaller effect of descending impulses (when they have any effect) may be due to the same factors that lead to their slower adaptation. Clearly the matter does not merit further discussion until more experimental results are available.

It is interesting to compare the effects of antidromic impulses on the rhythmic response of the nerve ending with those produced on the rhythmic discharge of a motor neurone when impulses are backfired into it; these have been studied by Eccles and Hoff [1932]. The effects appear very similar; in both cases the rhythm is "reset," and after an

antidromic impulse the recovery of the rhythmic structure is delayed by an amount determined by the position of that impulse in the rhythmic cycle. Eccles and Hoff were not able to vary the rhythmic rate over a wide range, as is possible with the sensory ending, but in some of their experiments the behaviour with changed rate was identical with that found here, that the absolute value of the delay alters in proportion to the cyclic interval, while in others differences occurred with only slight alterations in the rhythmic rate.

From the sensory endings no sign has been found of the grouped discharges following the antidromic impulse which were frequently observed by these authors from the motor neurone.

The similarity of behaviour of the motor neurone and sensory ending suggests that much of their behaviour may result from the common properties of polarized surfaces; this has been suggested above for the nerve ending from other considerations.

SUMMARY.

1. A method is described by which the impulses from single sensory nerve endings in mammalian muscle have been studied.
2. A new form of myograph assembly, which has been made for this work, is described.
3. Four distinct types of receptor have been identified. They are here designated A1, A2, B and C.
4. The rate of response of these receptors is roughly proportional to the logarithm of the tension on the muscle. Adaptation occurs very slowly.
5. Observations on the response to quick stretch and release suggest the end-organs containing A1 and A2 endings to have viscous elastic structure.
6. During active contraction the response of the A1 receptors ceases; they behave as if they lie "in parallel" with the contractile elements.
7. During active contraction resulting from supramaximal stimulation the response of the A2 endings accelerates, but if the stimulus is slightly submaximal it ceases. It is concluded that these endings lie in the muscle spindles and that only during supramaximal stimulation do the intrafusal fibres contract, and that when they do so they stimulate the A2 nerve endings mechanically.
8. From the behaviour of the A2 endings during twitch it is concluded that in soleus the intrafusal fibres are relatively more viscous than the ordinary muscle fibres.

9. The B endings have a high threshold and during contraction always behave as if they lie "in series" with the contractile elements. Their response depends only on the total tension on the muscle whether it be the result of passive stretch or active contraction.

10. The C endings lie in the fascia associated with muscles, they adapt rapidly, and only respond during movement of the muscle; they are few in number and probably not of great significance.

11. The time relations of impulses from A 1 receptors are slower than those from the A 2 and B receptors. They are thought to be travelling in smaller fibres.

12. Evidence is considered from which it is concluded that the A 1 response comes from the flower-spray endings of muscle spindles; the A 2 response from the annulo-spiral endings; the B response from the tendon organs of Ruffini.

13. The central effects of the impulses from these types of receptors are considered; it is thought that the A 1 response may be excitatory for the stretch reflex and the B response inhibitory. The function of the A 2 response cannot be decided from the present evidence.

14. When the circulation to the muscle is occluded, if the motor nerve be stimulated the excitability of the sensory nerve endings at first falls but later rises far above its normal level; finally in the absence of stretch a spontaneous discharge appears, its frequency rises to about 400 per sec. and after a minute or two it ceases and the ending is inexcitable until the circulation is released, when it rapidly recovers. This phenomenon is thought to be due to a breakdown of the nerve-ending mechanism. Theoretical aspects of this mechanism are considered.

15. The above effect is compared with the pain occurring in man when work is done by muscles with impeded circulation; the two have a number of common features. The possibility that the rapid discharge from stretch receptors may evoke pain is discussed.

16. The rhythmic mechanism of the end-organ is examined by observing the disturbance of rhythm brought about by antidromic impulses set up by electrical stimulation. The rhythm is "reset" by antidromic impulses which are followed by a delay the magnitude of which depends on the position of the antidromic impulse in the rhythmic cycle of the ending. Theoretical considerations are discussed and the behaviour of a nerve ending and motor neurone is compared; the effects produced by antidromic impulses are alike.

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STUDIES ON HISTAMINE HYPOTENSION.

By F. DOMENECH-ALSINA.

*(From the Institute of Physiology of the Barcelona School of Medicine.)**The effect of histamine on the circulation.*

A LARGE number of the results obtained in the investigation of the effect of histamine on the circulation of the cat cannot be applied to the dog. The blood-pressure of the dog does not exhibit the curve in three phases produced in that of the cat by a large dose, as described by Dale and Laidlaw [1918]. In the dog the fall of the blood-pressure is unbroken by a secondary rise, and the pressure remains low until spontaneous recovery sets in. In experiments with perfused organs the differences persist. Burn and Dale [1925] showed that the perfused organ of a dog quite readily gives an obvious vaso-dilator response to histamine, and that this dilator effect can still be demonstrated in an isolated preparation of the mesenteric artery and its branches, while a similar preparation from the cat shows nothing but vaso-constriction with histamine, as Dale and Richards [1918] had also shown earlier.

These results obtained by Burn and Dale, which clearly demonstrate the dilating effect of histamine upon the arterioles in the dog, confirm the differences observed in the living animals, and also the view that a general vaso-dilator effect is chiefly responsible for the depressor action of histamine in the dog. Ganter and Schretzenmayr [1930] are inclined to assume that the general effect of histamine on the circulation is due to arterial dilatation. Feldberg and Schilf [1930], surveying the published evidence, are also inclined to assume vaso-dilatation in the major circulation to explain the hypotensive action of histamine.

The suggestion of Mautner and Pick [1915], that the constriction of the hepatic veins found by them in the perfused liver of a dog and also in the intact animal, was the primary cause of the fall of blood-pressure, was disproved for the cat by Dale and Richards [1918], who demonstrated that the latency of the histamine depressor effect was shorter after intra-arterial injection, a little longer after injection into the jugular vein, and longer still after injection into the portal vein.

Finally, they showed that after extirpation of the alimentary canal, when the hepatic circulation was practically abolished, the fall produced by histamine persisted. Domenech-Alsina [1929] was able to prove that, in the dog also, the initial effect on pressure persists after the extirpation of the alimentary canal from the cardiac sphincter to the rectum.

THE EFFECT OF HISTAMINE ON THE DIGESTIVE GLANDS AND ITS IMPORTANCE.

Histamine increases the secretion of the digestive glands, as proved by various authors; salivary and pancreatic glands [Dale and Laidlaw, 1910], gastric glands [Popielski, 1920], intestinal glands [Koskowski, 1926], etc. Domenech-Alsina and Raventos [1929] suggest that the decrease of blood sodium found by them in the course of histamine shock, as well as the decrease of muscle chlorine, may be related to this hyperfunction of the digestive glands.

The experiments presented in this article support this view. Domenech-Alsina [1929] has pointed out the importance of the loss of plasma in the region of the alimentary canal, in the corpuscular concentration of histamine shock in the dog.

EXPERIMENTAL.

The object of these experiments is to investigate further the part played by the alimentary canal in the loss of the plasma seen in shock. This was not quite clear in our earlier experiments, owing to the increase of corpuscular content produced by the operation of extirpating the alimentary canal, which also played a part in the changes in the blood sodium and in the chlorine of the tissues. We also present experiments made with the object of investigating the mechanism of histamine hypotension in the dog, showing the various types of pressure curve with different methods of administration of histamine, the action of asphyxia and of large injections of fluid on histamine hypotension, the action of histamine on the capillary circulation of the dog's tongue, and the action of histamine on the decapitated animal.

General methods.

These experiments have been made on dogs anesthetized with chloralosan, with records of the blood-pressure in the femoral artery. We have used the histamine chloride of Hoffman-la-Roche, and all injections have been intravenous.

*Varying course of hypotension and recovery with varying rates
of administration of histamine.*

The intravenous injection of histamine brings about a sudden and pronounced fall in blood-pressure, to an average level of from 30 to 50 mm. Hg, which is maintained until spontaneous recovery sets in. For the sake of convenience we shall call this level "minimal hypotension." In normal circumstances, even with a dose of 1 cg./kg., we cannot obtain, with a single injection of histamine, a persistent state of hypotension. The cases of early death following such doses of histamine must be considered as accidental. Healthy dogs survive such an injection for a long period, during which they develop the series of alterations characteristic of shock. The course of recovery varies according to the mode of administration of histamine. After the injection of a single large dose, we have obtained curves analogous to those observed by Smith [1928]. There is a sharp initial fall; the pressure remains at the minimum for several minutes; then, after 10 or 15 minutes, begins to rise. This partial recovery, generally well marked, lasts for several hours. Then after several hours, sometimes as many as eight or nine, the pressure falls again until it reaches the hypotension minimum, remaining low until the death of the animal.

If the injection of histamine is begun very slowly, and the rate is increased progressively, it is possible to bring about a gradual hypotension without any sudden initial fall of pressure, although the amount of histamine injected soon exceeds the dose necessary to bring about a sudden hypotension in the non-prepared animal.

Exp. Dog, 7 kg. We began to inject the histamine at 11 h. 18 m. and then progressively increased the concentration of histamine in the solution to be injected.

	Blood-pressure
Initially	120
11 h. 40 m.	120
11 h. 53 m.	90
12 h. 10 m.	55
12 h. 35 m.	53
13 h. 32 m.	40

Up to this moment a total amount of 5 mg. of histamine per kg. had been injected.

Following Smith we may conveniently describe as "definitive hypotension" the terminal phase in which the blood-pressure, after having recovered partially during several hours, falls again to the minimum. This phase is specially well seen after the injection of a single dose of 1 cg./kg.

Effect of asphyxia on histamine hypotension.

Methods. Asphyxia by tracheal occlusion for 3 min. at different periods.

(1) Asphyxia before injecting histamine for the first time.

Exp. (a). Dog, 6 kg. The injection of 1.25 mg. of histamine, after 1 min. of asphyxia, is followed by a period of 2 min. during which asphyxia is continued and the pressure rises. When asphyxia ceases, the pressure rapidly falls.

Exp. (b). Dog, 8 kg. Injection of 8 cg. of histamine after 1 min. of asphyxia. The pressure does not fall for the moment; after 1 min. it falls a little, but is maintained well above the minimum. When asphyxia ceases, the pressure falls to the minimum.

(2) Asphyxia in the course of histamine hypotension. If spontaneous partial recovery of the pressure is still possible, asphyxia brings about a pronounced hypertension. In the definitive hypotension phase, when the animal has lost the power of spontaneous recovery, asphyxia does not produce a hypertensive response.

Exp. Dog, 7.5 kg.

11 h. 34 m. Injection of 7.5 cg. of histamine.

11 h. 56 m. to 21 h. 50 m. Pronounced recovery of pressure.

23 h. 50 m. Persistent hypotension.

23 h. 53 m. to 23 h. 55 m. Asphyxia. There is no rise of pressure.

23 h. 55 m. Injection of 1 mg. of adrenaline. Pressure rises to the initial figure.

24 h. 22 m. to 24 h. 24 m. Asphyxia. There is no rise of pressure.

24 h. 30 m. Injection of 1 mg. of adrenaline. Pronounced rise of blood-pressure.

Effect of a large injection of fluid on the histamine hypotension.

Methods. Injections of large quantities of physiological saline, during a short period of time, either into the jugular vein or into the right auricle or left auricle. In order to inject into either auricle, after opening the chest under artificial respiration, we introduced a wide tube through the corresponding auricular appendix, closing the thorax at once, leaving only an outlet for the tube.

(a) Effect of the massive injection of fluid on the initial hypotension.

Exp. Dog, 7.5 kg.

11 h. 15 m. to 11 h. 17 m. Injection of 250 c.c. of physiological saline into the jugular vein.

11 h. 16 m. Injection of 7.5 cg. of histamine.

The pressure falls rapidly to the minimum figure in spite of the injection of saline.

Exp. Dog, 8 kg. Thoracotomy. Placing of a tube in the right auricular appendix. After this the pressure is 75 mm.

18 h. 28 m. to 18 h. 31 m. Large injection of saline into the right auricle.

18 h. 30 m. Injection of 6 cg. histamine. The pressure falls rapidly to the minimum figure.

Exp. Dog, 16 kg. Thoracotomy. Placing of a tube in the left auricle. After this the blood-pressure is 100 mm.

From 18 h. 30 m. onwards. Large injection of saline into the left auricle.

18 h. 32 m. Injection of 8 cg. of histamine. Blood-pressure falls rapidly to the minimum figure (30 mm.).

(b) *Effect of large injections in different periods of the histamine shock.*

Exp. Dog, 7.5 kg. Repeated injections of physiological saline in different periods of the histamine shock (jugular vein).

11 h. 15 m. to 11 h. 17 m. Injection of 250 c.c. of physiological saline.

11 h. 16 m. Injection of 7.5 cg. of histamine.

12 h. 45 m. Injection of 250 c.c. physiological serum during 2 min.

15 h. 30 m. Injection of 250 c.c. physiological serum during 2 min.

19 h. Injection of 250 c.c. physiological serum during 2 min.

20 h. 45 m. Injection of 150 c.c. physiological serum during 2 min.

None of these injections of fluid immediately affected the blood-pressure. The spontaneous recovery of the blood-pressure was, however, greatly facilitated. At 23 h. 16 m. the dog's blood-pressure was still 120 mm. and the animal maintained a good peripheral temperature.

*Behaviour of the capillaries of the dog's tongue in the different periods of histamine hypotension*¹.

Methods. Record of the arterial pressure and direct examination of the capillary circulation of the dog's tongue after injections of variable doses of histamine.

In the dog's tongue the effects on the capillary circulation, of vascular relaxation on the one hand, and of the tendency of the organism to recovery of pressure on the other, are made very clear. The observations made with repeated injections of histamine are very interesting. A dose of 0.5 mg. per kg. gives rise to a capillary vaso-dilatation, which begins after a certain delay in relation to the sharp initial fall of the blood-pressure, since it begins 30 or 40 seconds after the pressure has reached the hypotension minimum; after some minutes (six or seven in *Exp.* 19) the capillaries again decrease in number and in volume. After a short while recovery of pressure begins. Fourteen minutes after the injection of histamine the capillaries are in the same condition as at the beginning; there is a notable recovery of pressure. In *Exp.* 19 the initial figure was 160 mm., the minimum figure in this experiment was 60 mm.; after 14 minutes the pressure had risen again to 115 mm.

Successive injections of histamine make the pressure fall again to the minimum figure. Renewed capillary dilatations become evident 45 or

¹ These observations made with the collaboration of J. Bofill were reported orally to the Societat de Biologia of Barcelona but have not been published (29th April 1932).

60 seconds after the animal has reached the hypotension minimum, but these disappear each time a new recovery begins. This is lower each time because the tendency to recovery is each time less. The vaso-dilatation following the administration of histamine is also less marked at each successive injection.

When histamine is injected continuously, but in doses sufficient to bring about a sharp initial fall in pressure, the state of capillary tonus follows a comparable course. Soon after the pressure has fallen to the minimum the capillaries begin to dilate. This capillary vaso-dilatation is transitory. In spite of the fact that the injection of histamine is going on, the capillaries soon begin to contract, and after some minutes are even more contracted than at the beginning. Rise of pressure starts soon after the capillary constriction has begun. On increasing the speed of injection the pressure falls and the capillaries again dilate.

After the injection of a single large dose (1 cg./kg.) the capillaries dilate, also with some delay in relation to the fall of pressure. In the recovery phase they again contract, becoming even more contracted than at the beginning of the experiment.

In the final period of hypotension, instead of a visible capillary vaso-dilatation there is emptiness of the visible capillaries in the tongue.

Part taken by the digestive tract in corpuscular concentration and in the blood changes of histamine shock¹.

Exp. Dog, 13 kg.						Blood-pressure	Hæmoglobin (Sahli)
17h. 15m.	120	65
17h. 20m.	Extirpation of the digestive tract from the cardiac sphincter to the rectum						
18h. 5m.	90	84
18h. 10m.	5 mg. histamine	40	—
18h. 13m.	40	86
18h. 28m.	60	—
18h. 30m.	5 mg. histamine	40	—
18h. 37m.	40	87
18h. 50m.	50	76
19h. 1m.	5 mg. histamine		
19h. 20m.	40	75
19h. 37m.	The animal dies						

In these earlier experiments the extirpation of the digestive tract was accompanied by a pronounced corpuscular concentration due to the fact that, as the extirpation began at the ileo-cæcal segment the separation of the greater part of the intestines from the circulation was somewhat

¹ This section, carried out partly in collaboration with J. Raventos, has been reported orally to the Societat de Biologia of Barcelona and has been included with the works of the Institut de Fisiologia.

retarded, and the intestine was partly filled up with fluid before being completely separated. For this reason the results were not quite clear. The corpuscular concentration, however, did not increase when shock was brought about by the histamine, but, on the contrary, it somewhat diminished. Figures of 75 for hæmoglobin (Sahli), in full histamine hypotension, are much lower than those found under normal conditions.

In later experiments, by rapidly extirpating the ileum from the beginning, we almost completely avoided the corpuscular concentration caused by the operation.

	Hæmoglobin		Hæmoglobin
<i>Exp. Dog, 8 kg.</i>	(Sahli)	<i>Exp. Dog, 10 kg.</i>	(Sahli)
Initially	110	17h. 30m. Initially	60
After extirpation of the digestive tract	115	18h. 50m. After the extirpation of the digestive tract	60
12h. 22m. to 13h. 15m. Repeated injections of histamine		19h. 20m. After repeated injections of histamine	50
13h. 16m.	90		

*Extirpation of the digestive tract from the pylorus to the rectum,
leaving the stomach intact.*

Under these conditions a corpuscular concentration takes place, which is less pronounced and takes longer to appear than in the normal animal. This corpuscular concentration is due to the loss of water into the stomach, which fills itself with liquid in the course of the experiment.

Dog, 20 kg.							Hæmoglobin
11h. 30m.	95
11h. 55m.	12h. 30m.	Extirpation of the intestine leaving the stomach intact					
12h. 45m.	100
13h.	Injection	2 mg.	histamine				
13h. 12m.	"	4	"				
13h. 20m.	"	6	"				
13h. 30m.	"	8	"				
13h. 35m.	100
13h. 40m.	Injection	10 mg.	histamine				
13h. 56m.	"	10	"				
14h. 15m.	"	20	"				
14h. 25m.	105
15h. 10m.	Injection	20 mg.	histamine				
15h. 30m.	105
15h. 40m.	Injection	15 mg.	histamine				
16h. 10m.	"	30	"				
16h. 25m.	"	20	"				
16h. 35m.	115
16h. 45m.	Injection	40 mg.	histamine				
17h. 10m.	"	50	"				
17h. 30m.	117
18h. 45m.	Animal dies						

Compare these figures with the following obtained in the intact animal with approximately the same doses of histamine.

							Hæmoglobin (Sahli)
Dog, 13 kg.							
11h. 30m.	85
11h. 31m.	Injection	1 mg. histamine					
11h. 40m.	"	2	"				
11h. 50m.	"	3	"				
12h.	"	4	"				
12h. 15m.	"	5	"	105
12h. 30m.	"	5	"				
12h. 50m.	"	10	"				
13h. 10m.	"	10	"	125
13h. 30m.	"	10	"				
13h. 50m.	"	20	"	135
14h. 10m.	"	20	"				

Part played by the digestive tube in the loss of muscle chloride and of blood sodium in the histamine shock.

The extirpation of the digestive tube prevents the loss of muscle chlorine which Domenech-Alsina and Raventos [1929] have described in histamine shock.

	Dog with digestive tube mg. p.c.	Dog without digestive tube mg. p.c.
Initial muscle chlorine	83	80.07
Chlorine after injections of histamine	44.7	81.80

The loss of sodium from the blood in the course of shock also takes place in the digestive tube, since after the total extirpation of the tube an injection of histamine is not followed by a decrease in sodium in the blood.

<i>Exp.</i> Dog, 10 kg.	g./L
Initial sodium in the blood	4.20
18h. to 18h. 50m. Extirpation of the digestive tube	
18h. 50m. to 19h. 10m. Repeated injections of histamine	
19h. 25m. Sodium in blood	4.10

This small variation in the blood sodium is of no significance when we realize that the blood sodium in the dog normally falls considerably half an hour after the histamine injection has begun.

After the extirpation of the intestine only, leaving the stomach intact, the blood sodium not only does not diminish, as a consequence of histamine shock, but increases.

<i>Exp.</i> Dog, 20 kg.	g./L
11h. 10m. Initial blood sodium	5.58
11h. 55m. to 12h. 30m. Extirpation of the intestine	
13h. to 13h. 30m. Repeated injections of histamine	
13h. 35m. Blood sodium	7.42
13h. 40m. to 17h. Repeated injections of histamine (The total exceeds 1 eg. per kg.)	
17h. 30m. Blood sodium	7.38

These results can be readily explained. The partial extirpation of the digestive tube does not prevent completely the loss of water and the consequent corpuscular concentration, as the loss of water continues through the stomach. But, on the other hand, as the loss of sodium is completely abolished by the elimination of the intestine, the blood sodium undergoes a concentration.

The action of histamine in the spinal dog.

In the trunk of a decapitated dog, with artificial respiration, the injection of histamine (1 mg./kg. in a single dose) was followed by a fall of pressure to the hypotension minimum. After some minutes the blood-pressure recovered spontaneously; on repeating the injection there was another fall of pressure to the minimum. Continuing the experiment, we asphyxiated the animal by suspending the artificial respiration: this was followed by a pressor response. The initial level was reached, a relatively low one, but sufficiently above that of hypotension minimum to show the effect of asphyxia clearly.

In the isolated trunk, also, this pressor response to asphyxia was absent in an advanced state of hypotension.

DISCUSSION.

From the fact that a single intense vaso-constrictor stimulus, such as that produced by asphyxia in the primary hypotension, or by adrenaline in all the phases of hypotension, can effect an increase of the blood-pressure, we may deduce the existence of an extensive vascular relaxation from the beginning of the histamine hypotension in the dog.

The immediate inefficacy of the injection of a massive quantity of liquid into the circulatory system also supports this view. Neither decrease of the blood volume, nor cardiac depletion, seems to play a direct part in producing the hypotension although the decrease of the blood volume will unfavourably affect the reaction against hypotension.

The significance of the observations on the capillary circulation of the dog's tongue is weakened by the fact that they are confined to a

localized condition. The phases of dilatation and contraction, compared with the blood-pressure curve, show clearly the phase of vascular relaxation brought about by histamine and the phase of constriction characteristic of recovery. We are inclined to believe, taking specially into account the delay of the capillary dilatation in relation to the fall of pressure, that the capillary dilatation is only a manifestation at the level of the capillaries of a general vascular relaxation. In the dog the capillary vaso-dilatation cannot be considered a fundamental factor in the cause of the hypotension.

The persistence of the effects of histamine in the decapitated animal shows that these effects are independent of the higher nerve centres.

My experiments as well as those made by Raventos and myself seem to indicate that the loss of chloride and sodium in the course of shock takes place into the digestive canal. With reference to the corpuscular concentration, the results described seem to indicate that the loss of fluid by this route is of considerable importance. Dale and Laidlaw [1919], however, observed that in the cat after the extirpation of the stomach, intestines, spleen and the consequent separation of the liver from the circulation, the injection of histamine was followed by an increase in corpuscular concentration similar to that obtained in the normal animal. We cannot, therefore, exclude the possibility that the different effect found by us in the dog may be accounted for by the different actions of the histamine on the hepatic circulation in the dog and in the cat. The phenomenon, described by Mautner and Pick [1915], of "*Lebersperre*" produced by histamine in the dog's liver, is not produced in the cat [Bauer, Dale, Poulsson and Richards, 1932]. It is possible, as suggested to me by Sir Henry Dale, that the effect of extirpating the digestive tract on the change produced by histamine in the corpuscular concentration may be at least partly due to the fact that this operation, by depriving the liver of the portal circulation, would prevent the marked oedema of the liver normally produced by histamine, which may be an important factor in the loss of plasma.

Therefore, and in spite of the fact that the loss of chlorine, and especially the exaggerated loss of sodium, inclines us to believe that the elimination of the plasma through the digestive tract must be very important, new experiments are necessary to determine the part played by the liver. Although we have observed several times that the digestive tract is filled with fluid in the course of the shock (the elimination of fluid matter by the anus is often copious), we have not made as yet any measurements of the actual quantities.

I believe that in the dog the existence of an important loss in the muscle capillaries can be excluded, in view of the lack of corpuscular concentration after the extirpation of the digestive tract, and also in view of the decrease of muscle chlorine in the course of the shock.

The possibility of obtaining a progressive curve of hypotension, without a sudden initial fall, by increasing progressively the speed of injection, proves that it is possible to abolish the initial histamine hypotension by superposing from the beginning the tendency of the organism to recover on the direct depressor action of histamine.

SUMMARY.

The study of asphyxia, of the effects of artificial increase of the blood volume, and of the capillary circulation of the dog's tongue, supplies arguments in favour of the assumption of an extensive vascular relaxation in histamine hypotension as seen in the dog.

Although the experiments here described strongly suggest that the loss of fluid at the level of the digestive tract is of predominant importance in the production of corpuscular concentration in the histamine shock of the dog, it is not possible to draw a final conclusion on this point without further experiments to eliminate a possible action of the "Leber-sperre."

Histamine shows the same reactions in the decapitated body of the dog as are observed in the intact animal.

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after removal of the uterus. The ferret was chosen because pseudo-pregnancy in this species has approximately the same length as true pregnancy [Hammond and Marshall, 1930], so that the necessary absence of fetuses would not complicate the study of the ovarian cycle.

II. MATERIAL AND TECHNIQUE.

Six animals were hysterectomized towards the end of anæstrus to determine the effects on the onset of the breeding season. Three of these were first-year animals and three were parous. Two others were subsequently hysterectomized, one 6 days after coitus, and the other at about the third week of pregnancy.

The uterus was removed through a medial abdominal incision about 2 in. long. The Fallopian tubes and the relevant vessels of the broad ligament were ligatured, as well as the top of the vagina. By this means the two cornua and the greater part of the cervix were removed with little bleeding. In one animal (FH 4) a fragment of uterus was found adherent to the Fallopian tube at autopsy. Our usual histological and other techniques were employed.

III. ŒSTRUS AND OVULATION.

The six ferrets hysterectomized during anæstrus all developed the characteristic œstrous swelling of the vulva during March-April. Two (FH 3, 6) were left unmated, one being killed after a short time on œstrus, and the other after 2 months of the usual persistent œstrus. Four (FH 1, 2, 4, 5) which were mated copulated normally. Two (FH 1, 2) of these which were left through a complete cycle came into œstrus again late in the season and once more copulated normally. The two ferrets (FH 7, 8) hysterectomized during pregnancy both came into œstrus later, and one (FH 7) which was mated copulated normally. In every case the swelling of the vulva subsided in the usual way after mating. So far as external indications were concerned, therefore, the occurrence of œstrus in the hysterectomized ferrets was perfectly normal.

The three animals killed on œstrus were seen on dissection to have large follicles in the ovaries. On histological examination one ovary of FH 6 was found to have four normal mature follicles between 1.35 and 1.5 mm. mean diameter. One ovary of FH 3 had two large follicles of 1.3 and 1.35 mm. mean diameter. The follicles of FH 8 were of similar size. These figures correspond with those given by Hammond and Marshall [1930] for the normal œstrous ferret. One large cystic follicle

was found in FH 8, but such structures are not uncommon in normal ferrets. The five hysterectomized ferrets allowed to copulate all ovulated, as determined by the subsequent finding of young, mature or regressing corpora lutea in the ovaries. FH 1 and FH 2 each copulated and ovulated twice at separate periods of œstrus. Hysterectomy in the ferret, therefore, does not affect the extra-uterine changes characteristic of œstrus. Judging by the number of ripe follicles in the animals killed on œstrus, and by the number of corpora lutea in the pseudopregnant animals, hysterectomy has no effect on the number of eggs ovulated at a time.

IV. PSEUDOPREGNANCY AND THE DEVELOPMENT OF THE CORPUS LUTEUM.

Table I shows that the hysterectomized animals include four killed at different stages of pseudopregnancy, and two used to determine the full length of pseudopregnancy. In addition, FH 7 and 8 give some indication of the length of pseudopregnancy after removal of the uterus in early pregnancy. There is no abnormality in the development of the pseudopregnant condition after hysterectomy, so far as can be seen in

TABLE I. Œstrus cycle in hysterectomized ferrets.

No. of animal	Date of hysterectomy	Condition at hysterectomy	Subsequent history before autopsy	Stage when killed
FH 1	23. ii. 32	Anœstrous	Œstrus-coitus-pseudopregnancy-œstrus-coitus	8 days <i>post-coitum</i>
FH 2	23. ii. 32	„	Œstrus-coitus-pseudopregnancy-œstrus-coitus	2 weeks <i>post-coitum</i>
FH 3	11. ii. 32	„	Œstrus (unmated)	8 weeks on œstrus
FH 4	16. ii. 32.	„	Œstrus-coitus-pseudopregnancy	4 weeks pseudopregnant
FH 5	21. ii. 32	„	Œstrus-coitus-pseudopregnancy	5 weeks pseudopregnant
FH 6	18. ii. 32	„	Œstrus (unmated)	Early in œstrus
FH 7	13. iii. 32	6 days p.c.	(Pseudopregnancy)-œstrus-coitus	2 days <i>post-coitum</i>
FH 8	11. iii. 32	Pregnancy ? 3 weeks	(Pseudopregnancy)-œstrus	On œstrus

our material obtained at 8, 14, 28 and 35 days *post-coitum*. The development of the corpora lutea, described below, is essentially normal. Of the available accessory organs, the vagina has no very characteristic change in pseudopregnancy, but the mammary glands of the hysterectomized animals underwent the development described by Hammond and Marshall [1930] for the normal animal.

The length of the two complete pseudopregnancies, calculated from the date of coitus to the start of the next vulval swelling, was 54 days (FH 1) and 45 days (FH 2). Hammond and Marshall [1930] give the duration of pseudopregnant changes as 5½ weeks and the time between

sterile coitus and the beginning of the next œstrus as 8 weeks. The periodicity of the hysterectomized animals was therefore essentially normal.

In the two animals hysterectomized during pregnancy the time elapsing before the onset of the next œstrus was irregular. In FH 7 swelling of the vulva began again at only 33 days *post-coitum*, while in FH 8 the time was 46 days after hysterectomy carried out at about 3 weeks pregnant.

Growth and regression of the corpus luteum. The earliest post-ovulation stage observed in these animals was in FH 7, killed 54 hours after coitus. One ovary contained five ruptured follicles (mean diameter 1.4 mm.), in two of which the ovulation point could still be distinguished. Masses of extravasated red blood corpuscles filled the cavities and could be seen among the growing lutein cells. Fibroblastic tissue was actively growing in from the theca interna. At 8 days after coitus (FH 1) there were well-formed corpora lutea, three of which had a mean diameter of 1.66 mm. These no longer had a central cavity, but a core of fibrous tissue. The lutein cells were well vascularized and showed an increase over those of FH 7; they tended to be larger at the exterior. After fixation in Flemming's fluid a light granulation of osmicated fat could be seen in the lutein cells and heavier fatty deposits in the theca interna. The corpora lutea of FH 2, 14 days after coitus, showed that growth had continued normally; two of them had a mean diameter of 1.93 mm. The increase was due to enlargement of the lutein cells; their nuclei showed little if any change. Many of the blood sinuses were dilated. Deposits of osmicated fat were seen in section, after Flemming fixation, as in FH 1, but the cytoplasm was darker, probably denoting a change or increase in the cell fats. At 4 weeks after coitus (FH 4) the corpora lutea were very large; the mean diameter of five was 2.3 mm. These were larger than those of a normal 3 weeks pseudopregnant ferret (mean diameter 2.03 mm.) and larger than the maximum size given by Hammond and Marshall [1930]. The full development had, therefore, been reached in the hysterectomized animal. In Flemming-fixed sections osmicated fat was abundant but still regularly distributed in fine granules. Groups of theca interna cells had persisted, but some had lost their fat granulation. At 5 weeks pseudopregnant (FH 5), the corpora lutea showed marked regressive changes; the mean diameters of two were 1.60 and 1.65 mm. respectively, giving a volume only one-third that of the corpora lutea at 4 weeks pseudopregnant. The lutein cells and nuclei had shrunk by comparison with FH 4 and with the normal 3 weeks pseudopregnancy.

In some corpora lutea there seemed to be a proliferation of the fibrous vascular tissue: one corpus luteum contained pigment. In the Flemming preparations the corpora lutea appeared to be much less fatty than in FH 4; the fat showed a fairly even disappearance from the edge of the section where the cytoplasm was colourless but with a light granulation of black osmicated fat. In the interior of the corpora lutea the cytoplasm was still brown, but comparatively free from black granules.

The regression of the corpus luteum appears to be rapid, to judge from the contrast between the 4 and 5 weeks pseudopregnant ferrets. During the last part of pseudopregnancy and the following pro-œstrus, existing corpora lutea dwindle to small patches of fibrous tissue containing pigment and a little fat. Such degenerate remains can be found in FH 7, FH 1 and FH 2, at 2, 8 and 14 days after the next ovulation.

V. SUMMARY.

Hysterectomy in the ferret during anœstrus does not affect the occurrence or morphological changes of œstrus, ovulation or pseudopregnancy in the next breeding season. During the time covered by the experiments no evidence was obtained of prolongation of luteal activity or of ovarian degeneration.

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THE ACTION OF STRYCHNINE ON HERING-BREUER REFLEXES.

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IN 1911 Owen and Sherrington were unable to reach a decision on the question whether strychnine acts by transforming the process of central inhibition into one of central excitation or by selectively raising the responsiveness of reflex arcs to excitatory, but not, or not to the same extent, to inhibitory influences. On either view a ready explanation is found for the familiar reversal of inhibitory reflex effects when these are evoked by stimulation of a large nerve trunk containing a mixture of afferent fibres, the majority of which are potentially inhibitory, while a few are potentially excitatory, with respect to a given test muscle.

More than ten years later, Bremer [1922, 1925] found that the inhibitory effects of stimulating the cortex of the anterior lobe of the cerebellum are neither reversed nor diminished by strychnine. This has been confirmed by Miller [1926]. Bremer was therefore led to the conclusion that strychnine "reversal" is an expression of the stimulation of mixed afferent nerves, and that the action of the drug is not to convert the central process of inhibition into excitation but merely to increase enormously the excitability of motor arcs. On a purely inhibitory reflex strychnine is devoid of influence. In support of his contention he quotes the finding of Magnus and Wolf [1913] that inhibition of triceps brachii and of vastocruureus in tonic neck and labyrinthine reflexes is not reversed by strychnine.

Further examples of the absence of reversal in reflexes in which there is reason to believe that a homogeneous set of receptors is alone involved have since been brought to light. Liddell and Sherrington [1925] noted the continued inhibition of the stretch reflex of quadriceps on passive stretch of a knee flexor after the administration to decerebrate cats of 0.25 mg. of strychnine per kg. body weight. Less than one-third of this dose will cause "reversal" on stimulation of a bared afferent nerve.

Cooper and Creed [1927] obtained similar results when studying the relaxation of quadriceps evoked reflexly by active contraction of hamstrings. The work of Matthews [1931] makes it probable that the receptors involved in the latter experiments were muscle spindles.

The mode of action of strychnine becomes of considerable importance when one reflects that the conversion of central inhibition into central excitation would, if true, form an important datum in any discussion of the intimate nature of these two processes. It occurred to us that a simple method of providing additional evidence would be to test the action of the alkaloid on the relaxation of the inspiratory muscles which results from distension of the lungs. This vagal reflex was first described as a feature of "the self-regulation" of respiratory movements by Hering and Breuer [1868] and has been repeatedly confirmed by subsequent workers. It would seem to offer an unusually good opportunity of applying a normal adequate stimulus to a presumably homogeneous set of receptors. The diaphragm-slip technique of Head [1889] provided a suitable method of recording the movements of an inspiratory muscle during normal breathing.

Seemann [1910] noted that after giving rabbits strychnine "expiratory spasm from inflation of the lungs is much more powerful than before." He did not, however, record the movements of inspiratory muscles, and in other respiratory reflexes describes the occurrence of strychnine "reversal."

METHOD.

Rabbits have been used for all experiments on account of the ease with which their powerful diaphragm slips can be isolated for recording. Under deep anaesthesia (ether), Head's technique for preparing a diaphragm slip has been closely followed. That of the left side only was used. Its upper end was fixed to the chest wall by passing a loop of thread through a button on the abdominal surface of the diaphragm and knotting the ends in front over one of the lower costal cartilages. The lower end of the slip was left attached to part of the ensiform cartilage.

In the left hindlimb the nerve to hamstrings was cut. The sciatic nerve in this limb was also cut just above the knee and its central end prepared for stimulation. All movements of the ankle were thereby prevented, and active extension only was possible at the knee. Both carotid arteries were ligated and a tracheal cannula was inserted. The animal was then decerebrated through the midbrain by the trephine method and the anaesthesia discontinued.

Two hours later, when the ether had been blown off in the course of natural breathing, the preparation was transferred to the recording apparatus. Here it was tied supine. Movements of the fixed end of the slip were prevented by clamping the sternum and lower costal cartilages of the right side and rigidly attaching the clamp to the table top. The piece of ensiform cartilage into which the other end of the slip was inserted was connected by thread with a light lever writing on smoked paper. Care was taken that the slip had a free pull, clear of the liver and other obstructions. Except when observations were actually being made, it was packed in warm swabs wrung out of saline.

Artificial inflation or deflation of the lungs was brought about by one of the experimenters blowing or sucking through the tracheal cannula. The cannula was connected by a T-piece with a mercury manometer fitted with a float carrying a writing point. By this means the changes of intrapulmonary pressure were signalled on the moving smoked paper. A time marker recorded seconds.

GENERAL OBSERVATIONS.

In good preparations it was easy to obtain satisfactory records from the diaphragm slip of normal inspiratory contractions and expiratory relaxations. Very occasionally no movements could be observed although the animal appeared to be breathing normally. In these cases the nerve supply or blood supply of the slip had probably been damaged during the operation [cf. Head, 1889]. After successive administrations *per venam* of a 0.02 p.c. solution of strychnine hydrochloride, the movements thus recorded became more ample. We have no precise data as to the extent of this increase in the excursions, because the lever was detached from the slip while each injection was being made. But it was commonly well marked, and, for convenience in recording, the magnification of the excursions of the writing point was progressively diminished during an experiment. Wood and Cerna [1892], Impens [1899], Biberfeld [1904], and Cushny [1913] all agree that strychnine greatly increases the respiratory exchange. The last named, however, using both anæsthetized and decerebrate rabbits, differs from the others in finding the rate only of the breathing to be affected, while the depth is unchanged or even diminished. Our findings do not support him. Initially, the breathing of our preparations was often at as high a rate as 100 per min. Sub-convulsive doses of strychnine caused no increase, and sometimes a decrease, in this frequency. Doubtless the result might have been different on the intact or anæsthetized animal with a slower rate of breathing.

At the beginning of an experiment, stimulation of the prepared central end of the left sciatic nerve with weak induction shocks caused that knee, if tonically extended, to relax. Flexion of the hip regularly occurred and was frequently followed by powerful movements of progression in which all the four limbs participated. When strychnine had been injected intravenously in amounts exceeding 0.1 mg. per kg., similar stimulation of the sciatic nerve evoked obvious contraction in quadriceps extensor.

Between successive injections of strychnine the effects on the diaphragm slip of inflation and deflation of the lungs were recorded. The administration of the drug was continued until death supervened. The lethal dose in our preparations has usually been between 0.15 and 0.2 mg. per kg. body weight. Once opisthotonus and death have resulted from 0.1 mg. per kg., and once a dose of 0.2 mg. per kg. failed to kill while 0.225 did so. In the intact rabbit Maurel [1908] found 0.5 mg. per kg. *intra venam* to be always fatal, and 0.2 mg. to be never fatal. Our lower figures are probably attributable to the enhanced reflex excitability of decerebrate as compared with intact animals, and their consequent oversensitiveness to strychnine [cf. Cushny, 1913].

Our experience agrees with the statement of Poulsson [1920] that death is ushered in by only a single generalized convulsion, comprising opisthotonus and maintained extension of all the limbs. With sub-lethal doses there is, of course, abundant evidence of increased excitability, *e.g.* to blowing on the skin, and widespread convulsive jerks are readily induced. Very occasionally we have seen recovery from opisthotonus of several seconds' duration, but it has not in these cases been accompanied by full extension of the limbs.

EFFECT OF INFLATION OF THE LUNGS.

The effect of raising the intrapulmonary pressure by 1-3 cm. Hg is cessation of respiratory movement with relaxation of the diaphragm slip. Our findings agree with Head's in that the inhibitory pause may be preceded by an inspiration if the inflation is made suddenly. Thereafter the cessation of movement has generally, in our experience, been maintained throughout the longest periods of inflation that we have used (7 sec.).

The relaxation of the slip is no less marked after maximal doses of strychnine. Thus, to quote one of several similar experiments, 0.9 c.c. of a 0.02 p.c. solution of strychnine was injected into the femoral vein of a rabbit weighing 1800 g. Stimulation of the central stump of the left

sciatic nerve by weak induction shocks then evoked extension of the left knee, often followed by galloping movements. But even after two subse-

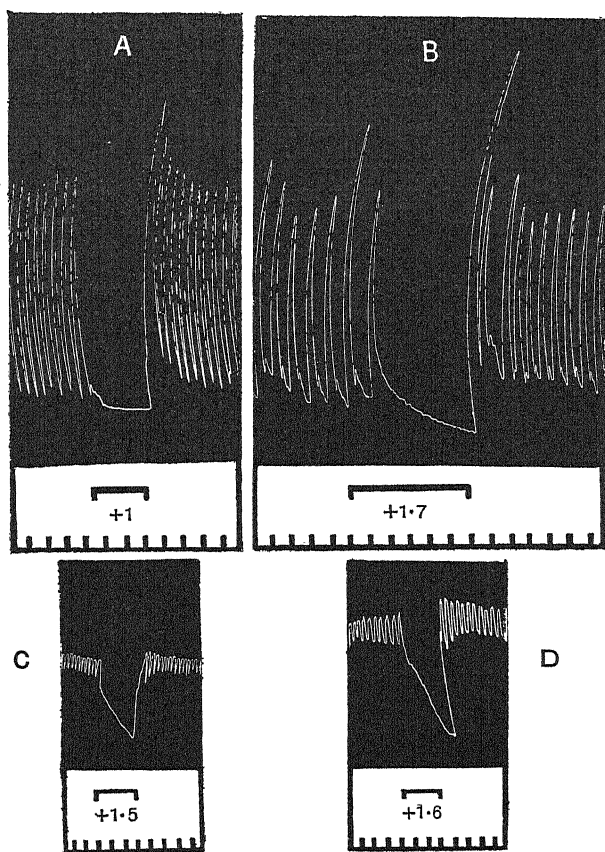


Fig. 1. Tracings from diaphragm slip of decerebrate rabbit. Inspiratory contractions upwards; expiratory relaxations downwards. The signal indicates the duration and the extent of artificial inflations of the lungs. The time marker gives seconds. A. Rabbit weighing 1800 g. 3 hours after decerebration. Intrapulmonary pressure raised by 1 cm. Hg. B. The same animal an hour later, after being given 0.17 mg. of strychnine per kg. body weight. Further 0.03 mg. was fatal. C. Another rabbit weighing 2400 g. Interval after decerebration 2½ hours. The diaphragm is evidently not fully relaxed in the expiratory phase of normal breathing. D. The same animal an hour later, after being given 0.2 mg. of strychnine per kg. body weight. Further 0.025 mg. was fatal.

quent injections, each of 0.3 c.c., the slip relaxed on inflation of the lungs (Fig. 1). A further 0.3 c.c. caused opisthotonus and death. Coincident with inhibition of the slip, contraction of the abdominal muscles has often

been noticed. This is obviously exaggerated under the influence of strychnine.

Although cessation of all respiratory movement has usually occurred both before and after giving strychnine, in one experiment the cessation appeared not always to be complete following very large doses of the drug. But even then the pauses between successive inspirations were much prolonged, and the level of the writing point in the expiratory position was slightly, and in the inspiratory position greatly, reduced. This isolated observation can be explained either by increased sensitivity of the poisoned respiratory centre to its normal stimuli (of which we have already produced evidence), or, less probably, by analogy with Langley's finding [1913] that vagal vaso-motor reflexes may be paralysed by giving a rabbit enormous doses (2 mg.) of strychnine. Scott [1925] has shown, by local application of the drug to the floor of the fourth ventricle, that this is due to a blocking of the reflex path in the medulla oblongata.

Be this as it may, there seems no room for doubt that, even when apparent reversal of the effect of sciatic stimulation is well marked, strychnine causes no reversal of the normal inhibitory relaxation of the diaphragm elicited by inflation of the lungs. The action of the drug therefore cannot be to convert central inhibition into central excitation.

EFFECT OF DEFLATION OF THE LUNGS.

Although the few observations to be reported under this heading have little bearing on our main theme, they seem worthy of record as helping to clear up certain points which have recently been raised in regard to respiratory reflexes. The intrapulmonary pressure has usually been lowered by about 3 cm. Hg by sucking air from the tracheal cannula; sometimes much larger and sometimes much smaller differences of pressure between the outside of the thorax and the pulmonary air spaces have been established.

No attempt will be made to deal fully with the extensive literature on the subject. This has lately been well summarized by Hammouda and Wilson [1932] and by Sharpey-Schafer [1932].

(1) *Influence on the tonus of the diaphragm.*

Deflations of from 0.8 to 6.5 cm. Hg have invariably been accompanied by a rise of tonus as evidenced by a higher level in the record traced by the diaphragm slip. The extent of this effect varies considerably in different preparations. As a rule it is much less marked after large

doses of strychnine, perhaps because the "normal" tonus of the inspiratory muscles is then raised almost to maximal degree.

Hammouda and Wilson [1932] disagree with Hering and Breuer, with Head, and with many other workers whom they quote, in finding no indication of any active inspiratory response to collapse. Their failure to observe the reaction is not surprising since the method they used gives no information regarding the condition of individual muscles. Collapse was induced by raising the pressure of the air surrounding the body of the animal (dog) by amounts varying from a few mm. to 90 mm. Hg, while the trachea communicated directly with air at atmospheric pressure. The records indicate the volume of air in the lungs at any given moment. With raised external pressure this volume may be expected to remain below normal even though the inspiratory muscles are contracting in a vain attempt to counteract the deflation. Our observations confirm the classical view that there is, in fact, an inspiratory response to deflation.

(2) *Influence on the rhythm of respiration.*

On causing collapse of the lungs by puncture of the pleuræ, Hering and Breuer and later Head reported long-lasting cessation of respiratory movement with spasm of the diaphragm. In one of Head's tracings from a diaphragm slip there is marked slowing of respiratory movement during closure of a rabbit's trachea at the end of normal expiration. Sharpey-Schafer and Bain [1932] have also published records showing a diminished frequency of respiration on closing the tracheæ of narcotized dogs at the height of the expiratory phase of normal breathing. On the other hand, their tracings of respiratory movements on lowering the intrapulmonary pressure by connecting the trachea with a large reservoir of air 1-2.2 cm. Hg below atmospheric pressure exhibit a quickening in the rate of breathing. Similarly Hammouda and Wilson, by the method already described, find that in dogs "collapse of the lungs beyond the normal position of equilibrium is accompanied by an increase in the rate of breathing proportional to the diminution in lung volume," and that this effect is abolished by section of both vagi.

At first sight, the former conclusions appeared to be confirmed, and the latter to be contradicted, by our observations. We have nearly always found slowing, though not complete stoppage, of diaphragmatic movement as the result of sucking air from the lungs of rabbits (Fig. 2). Strychnine has generally accentuated the effect. Further examination of the records, however, shows that the cases in which this occurs are those in which the intrapulmonary pressure has been reduced by more

than 2 cm. Hg (in one case 1 cm. gave slight slowing). Reductions of from 0.8 to 1.6 cm. Hg have usually resulted in a quickening of the rate even when greater reductions cause slowing. In the experiments on

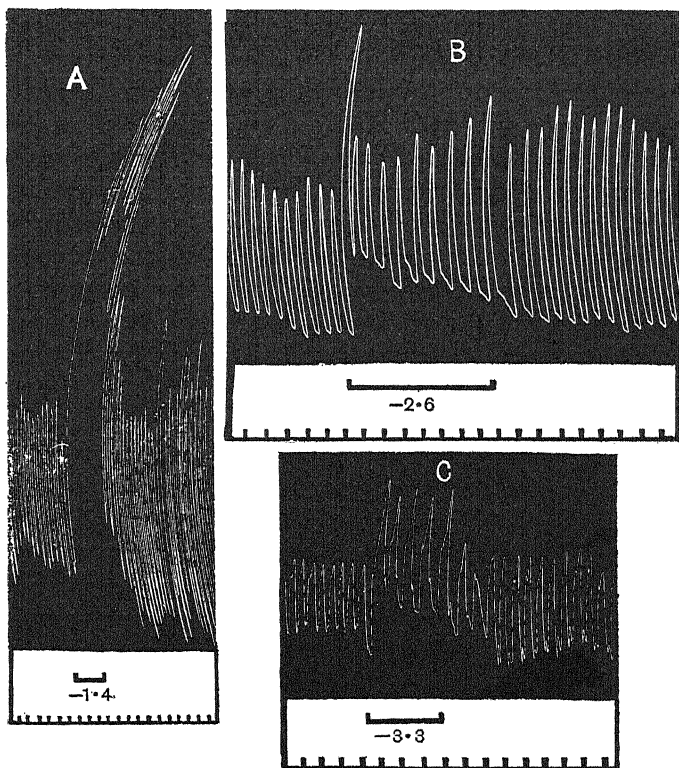


Fig. 2. Tracings showing the effect of deflation of the lungs. A. Rabbit weighing 2550 g. decerebrated 3 hours before. (It has been given 0.05 mg. of strychnine per kg. body weight.) Intrapulmonary pressure lowered by 1.4 cm. Hg. The frequency of the breathing is scarcely affected. The same reduction of pressure before strychnine caused definite quickening. Marked increase in tonus of the slip. B. Rabbit weighing 1800 g. decerebrated 4 hours before. (It has been given 0.13 mg. of strychnine per kg. body weight.) Reduction of intrapulmonary pressure by 2.6 cm. Hg causes obvious slowing. C. Rabbit weighing 1900 g. decerebrated 3 hours before. (No strychnine has yet been administered.) The frequency of the breathing is diminished and the amplitude of the excursions is increased by the deflation.

which Hammouda and Wilson base their conclusion that collapse induces increase in the rate of breathing, the excess of external over intrapulmonary pressure appears not to have exceeded 1.4 cm. Hg.

It therefore looks as though the influence of deflation on rhythm depends upon the extent of the deflation. Small deflations evoke increase, and large deflations evoke decrease, in the rate of breathing. That the latter is not due to absurdly intense stimulation is indicated by the facts (1) that our deflations were induced by volitional contractions of the inspiratory muscles of the experimenter, and (2) that occlusion of the trachea at the end of normal expiration undoubtedly occasions slowing. Whether the opposed effects are due to stimulation of two distinct sets of vagal endings; or to differences in the relative preponderance of vagal influences (*a*) on Hering-Breuer reflexes and (*b*) on the inherent rhythmicity of the respiratory centre [cf. Hammouda and Wilson, 1932]; or to the different effects of vagal and proprioceptive reflexes [see Boothby and Berry, 1915; Fleisch, 1928; Hammouda and Wilson, 1932; Sharpey-Schafer and Bain, 1932], we have no evidence. The decerebrate rabbit is so delicate a preparation that it would almost certainly die were its vagi divided. Study of other workers' results, however, leaves little room for doubt that there are afferent paths for both responses in the vagi. The matter is of some academic interest and would be worth pursuing further. But in ordinary circumstances a difference between intrapulmonary and external air pressures, with consequent alteration in the mean state of expansion of the lungs, must be of rare occurrence. The investigation would probably throw little light on the normal regulation of the frequency of respiration.

(3) *Influence on the amplitude of diaphragmatic contractions.*

The changes in amplitude resulting from pulmonary deflation are not, in our experience, constant. When the rise in tonus has been great, there has generally been a tendency to maintained contraction, and the respiratory excursions of the lever have been smaller than normal. On the other hand, when tonus has been little affected, not only are the excursions less frequent than normal, but their amplitude is often increased (cf. Head's Pl. 1, Curve viii).

With the dog's trachea closed at the end of expiration, Sharpey-Schafer and Bain [1932] record diminished movement of the epigastrium. This, however, should not be regarded as conclusive evidence of diminished diaphragmatic activity. If the force of the rhythmical contractions remained unaltered, the epigastrium would still be expected to move less than it normally does. For movement would now be opposed by the lowering of intrapulmonary pressure consequent on contraction of inspiratory muscles with no entry of air into the lungs. Nor is it

justifiable, in our opinion, to draw quantitative conclusions regarding the degree of activity of the inspiratory muscles as a whole from observations, during closure of the trachea, of the respiratory waves on a tracing of the blood-pressure. These waves depend mainly, it is thought, on changes in the mediastinal pressure, and with equal inspiratory efforts such changes will clearly be greater when the trachea is closed than when it is open.

SUMMARY.

1. Relaxation of a rabbit's diaphragm slip, evoked by inflation of the lungs, is never replaced by contraction as a result of administering strychnine. This finding supports Bremer's conclusion that the action of strychnine is not to convert central inhibition into central excitation, but to facilitate the passage of excitatory processes through reflex arcs.

2. Under strychnine, the amplitude of respiratory movements of the diaphragm is increased in the decerebrate preparation. The frequency is not increased.

3. The lethal dose of strychnine for the decerebrate rabbit is usually between 0.15 and 0.2 mg. per kg. body weight.

4. Deflation of the rabbit's lungs causes increased tonus of the diaphragm slip. When the intrapulmonary pressure is reduced by more than 2 cm. Hg the breathing becomes slower than normal; with smaller reductions of pressure, quickening occurs. Deflation has a variable influence on the amplitude of diaphragmatic contractions. These effects are compared with the findings of other workers who have often reached mutually contradictory conclusions.

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ERGOTAMINE AND THE EFFECT OF ADRENALINE ON BLOOD LACTATE.

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It is now well established that adrenaline hyperglycæmia is inhibited by adequate doses of ergotamine or ergotoxin. Further, the rate of fall of blood sugar after the injection of insulin is much more rapid if ergotamine has perviously been administered, a fact which could be inferred from the previous statement. It is also well established that adrenaline injection leads to a discharge of lactic acid into the circulation from an accelerated breakdown of muscle glycogen, the degree of rise in blood lactate depending upon the ability of the liver to resynthesize it to glycogen. If we regard the action of adrenaline on liver glycogenolysis as of the nature of a motor sympathetic effect, then the inhibitory action of ergotamine is in accordance with the usually accepted view that this substance is a paralyisant of the motor side of the sympathetic. The question arises as to whether the peripheral action of adrenaline on muscle glycogenolysis is of a similar nature and whether it can be inhibited by ergotamine. This matter has recently been examined by Nitzescu and Munteanu [1932], and in view of their findings we will present our own on this subject. These authors injected 2-2.5 mg. ergotamine tartrate per kg. into unanæsthetized rabbits and found that, after the subcutaneous injection of adrenaline, there was inhibition of the normal hyperglycæmia but the blood lactate rose as usual. They took the blood samples at hourly intervals from the heart. These results suggest that the mechanism of peripheral release of lactic acid by adrenaline is of a totally different nature from that of the release of glucose from the liver. The possibility of such a dual action of adrenaline might indeed be inferred from the peculiar difference in behaviour exhibited by glycogen in the liver and in the muscles, the former yielding glucose and the latter lactic acid. The matter might, perhaps more simply, be considered as due to differences

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in the properties of the enzymes of the liver and the muscles, or perhaps even as due to differences in structure in the glycogen when found in these different situations. The establishment of such a difference in behaviour towards ergotamine seems of some importance.

Now the above-mentioned authors used unanæsthetized rabbits, and we also attempted this with similar doses of ergotamine. To obtain blood samples from the peripheral vessels in such animals after the intense vascular constriction induced by ergotamine is impossible. Further, the injection is followed in a short time by intermittent and violent tremors, and the animal reacts so violently to manipulation that it was felt that any results thus obtained were suspect. Puncture of the heart for blood is not difficult, but to obtain serial samples in this way over considerable periods is likely to lead to hæmorrhages and death. Having regard to these considerations it was felt that the anæsthetized animal offered a more reliable approach. The difficulty thus introduced, however, is the anæsthetic, and the recent work of Murphy and Young [1932] indicates how great caution is necessary in interpreting results on anæsthetized animals in the matter of carbohydrate metabolism. Experiments will, however, be given showing the influence of the anæsthetics used.

EXPERIMENTAL.

Blood sugar was estimated by Maclean's method. For the estimation of blood lactate, the blood was obtained by means of a cannula inserted in the common carotid artery. The blood was allowed to run into a small graduated cylinder to the 2.5 c.c. mark and no anti-coagulants were used. From this 2 c.c. were pipetted into a centrifuge tube containing 14 c.c. distilled water and 2 c.c. $\frac{2}{3} N$ H_2SO_4 and 2 c.c. 10 p.c. sodium tungstate were added; the whole was then stirred with a glass rod, and after about 30 min. was centrifuged at about 3000 r.p.m. for from 3 to 5 min. In this way 14 to 15 c.c. of protein-free fluid are obtained. For the estimation of lactic acid 10 c.c. of this are transferred to a graduated 100 c.c. flask, 4 c.c. 20 p.c. $CuSO_4$ and 4 c.c. 10 p.c. $Ca(OH)_2$ added, and after about 20 min. the volume is made up to 100 c.c. with distilled water. Filtration of this yields about 85–90 c.c. of fluid, and of this 80 c.c., representing 0.8 c.c. blood, were used for the oxidation by the method of Friedmann, Cotonio and Shaffer [1927]. Using solutions of pure zinc lactate yields of 95–98 p.c. of the theoretical are always obtainable by this method for quantities of lactic acid of the same order as those met with in blood.

The effect of adrenaline in the anæsthetized animal.

Since amytal was largely used in these experiments it is necessary to consider whether the typical effects of adrenaline are produced when anæsthesia is induced with this substance. The following experiments deal with this matter.

(a) Rabbit. 3.5 kg. 24 hours without food. Amytal 180 mg./kg. intraperitoneally. Blood obtained from the left common carotid by cannula. Adrenaline hydrochloride 1 mg. at time indicated.

Time (min.)	0	10	28	43	70	103	145
Blood sugar mg./100 c.c.	104	Inj.	154	156	231	222	266
Blood lactate mg./100 c.c.	11	—	23	34	60	78	74

(b) Rabbit. 4.0 kg. 24 hours without food. Ether anæsthesia. Blood from jugular veins. Adrenaline 1 mg. subcutaneously at time indicated.

Time (min.)	0	15	25	60	105	155	195
Blood sugar mg./100 c.c.	98	127	Inj.	150	211	216	164
Blood lactate mg./100 c.c.	133	114	—	138	120	163	218

In (a) we see the unequivocal effect of adrenaline on the blood sugar and lactate during the amytal anæsthesia. In (b) the asphyxial action of ether makes itself manifest by an immediate rise in blood lactate, but the adrenaline can still further raise the lactate, indicating that the adrenaline effect is something more than local oxygen want.

The following experiments were carried out on cats.

(c) Cat. 2.6 kg. 24 hours without food. Amytal 250 mg. intraperitoneally. Cannulae in femoral vein and left common carotid. Slight spasms during the operation. Adrenaline 1 mg. intravenously at time given.

Time (min.)	0	27	32	52	77	87
Rectal temp. ° C.	38.5	39.2	—	40.0	41.0	—
Blood sugar mg./100 c.c.	152	181	Inj.	408	306	Died
Blood lactate mg./100 c.c.	36	16	—	74	99	—

The fulminating effect of adrenaline when introduced in very large doses is here clearly seen. The so-called adrenaline fever occurred in very marked degree. In the following experiment it is shown in how small a dose the effect of adrenaline is still perceptible.

(d) Cat. 2.55 kg. 24 hours without food. Amytal 300 mg. intraperitoneally. Cat anæsthetic in 3 min. Isotonic saline 30 c.c. containing 0.02 mg. adrenaline infused into the right femoral vein at a rate of 1 c.c. per min. Blood taken from the left carotid artery. Two control samples were taken 34 and 49 min. after the amytal injection and before the adrenaline infusion.

Time after beginning of infusion (min.)	0	0	6	12	20	30	65
Blood sugar mg./100 c.c.	79	87	111	87	95	104	139
Blood lactate mg./100 c.c.	13	13	12	16	16	20	23

This rate of infusion of adrenaline is the lowest (0.00026 mg. per kg. per min.) we have found to be followed by a demonstrable change in

sugar and lactate in the blood. That amytal does not in the times of the above experiments appreciably affect either blood sugar or lactate has been shown by several authors.

In the next experiment it is shown that amytal does not prevent the typical effects of adrenaline injected subcutaneously in cats.

(e) Cat. 2.9 kg. 24 hours without food. Amytal 400 mg. intraperitoneally. Cat anæsthetic in 5 min. Procedure as in former experiments. Adrenaline 1 mg. injected subcutaneously at time given.

Time (min.)	0	23	35	103	128	158
Blood sugar mg./100 c.c.	155	103	Inj.	250	303	238
Blood lactate mg./100 c.c.	20	23	—	30	50	69

In a similar experiment on a cat under urethane anæsthesia (2.5 g.) the blood lactate, after the intravenous injection of 0.5 mg. adrenaline, rose from 68 mg. to 146 mg./100 c.c. in 1 hour. It may be regarded as certain, therefore, that these anæsthetics allow of a large and rapid effect of adrenaline.

The effect of ergotamine on the above action of adrenaline.

The ergotamine was used either in the form of ergotamine tartrate or in the readily soluble form of ergotamine methan sulphonate (both kindly supplied by Messrs Sandoz).

The injection of large doses of ergotamine into anæsthetized cats is followed in a few minutes (sometimes seconds) by a cessation of respiration. It is therefore necessary to work with artificial respiration through a tracheal cannula. In some of the experiments to be described the respiratory pump was used from the start, and it was in these that the most unequivocal results were obtained. The rate and depth of inflation of the

TABLE I.

Cat. 5 kg. 24 hours without food. 760 mg. amytal intraperitoneally. Anæsthetic in 10 min. Cannulæ in trachea and in left carotid. Artificial respiration from the start.

Time min.	Blood		Rectal temp.
	Sugar mg./100 cc.	Lactate mg./100 c.c.	
0	222	22	38.0
22	Ergotamine tartrate 5.5 mg. intravenously.		
35	216	—	37.8
55	Adrenaline 1 mg. subcutaneously.		
85	177	24	38.5
115	166	20	39.0
145	227	20	39.0
175	259	24	39.5
205	256	24	—

Liver glycogen at end of experiment 6.3 p.c.

lungs were carefully regulated so as not to produce over-ventilation. Adrenaline injections were generally not commenced until the paralysis of the dilatores pupillæ was very well marked. It has been found that the pupil on the side on which the carotid artery was ligated for blood sampling was never so markedly constricted after ergotamine as on the non-ligated side. The animal was always kept on a warmed table and the temperature always recorded from the rectum.

The initial high blood sugar in this experiment was due to a short period of rather poor respiration before the respiratory pump was attached. It is to be observed that the blood sugar fell after the adrenaline injection before commencing a slow rise. But throughout the 2½ hours after the adrenaline injection there was no significant change in blood lactate. Indeed it would appear that a slight residual effect of the adrenaline on the blood-sugar level may occur without any accompanying effect on the lactate.

TABLE II.

Cat. 24 hours without food. Amytal 300 mg. intraperitoneally. Anæsthetic in 5 min. Cannulæ in left common carotid, left femoral artery and vein. Blood-pressure recorded from femoral artery.

Time min.	Blood		Rectal temp.	Remarks
	Sugar mg./100 c.c.	Lactate mg./100 c.c.		
0	159	32	38.5	
10	Ergotamine methan sulphonate 5 mg. in 5 c.c. water intravenously. Respiration stopped almost at once. Artificial respiration put on at once. Pupils normal.			
35	213	35	39.0	Pupils: L. 4 mm., R. 2 mm.
54	Adrenaline 0.4 mg. intravenously.			Effect on blood-pressure in Fig. 1.
66	Adrenaline 0.6 mg. subcutaneously.			
80	224	43	38.5	Pupils: L. 2 mm., R. 1 mm.
114	191	41	—	Tremors in forelimbs.
120	Adrenaline 0.3 mg. intravenously.			Effect on blood-pressure in Fig. 2.
140	Adrenaline 0.3 mg. intravenously.			Effect on blood-pressure in Fig. 3.
144	249	44	39.0	
174	216	52	—	
180	Adrenaline 0.3 mg. intravenously.			Effect on blood-pressure in Fig. 4.
200	211	59	39.0	

Judged from the effects on the blood-pressure it is seen that the paralysis of the vaso-constrictors was not complete except for a relatively short period. Only in Fig. 2, taken after a dose of 0.3 mg. adrenaline 110 min. after the ergotamine, do we find an almost complete failure of a pressor response. At each of the other injections a definite rise in blood-pressure occurred, although the response was atypical, consisting of an initial sharp rise, then a fall, and then a prolonged rise. Fig. 4 shows very well the sustained rise in pressure (46 mm. Hg). From the 120th min.

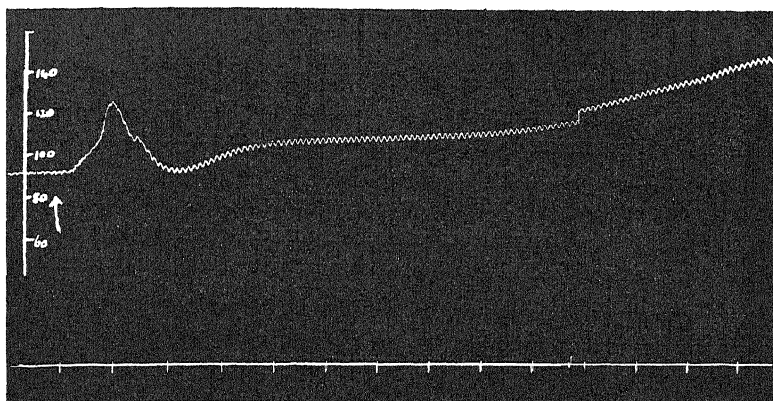


Fig. 1. 0.4 mg. adrenaline at arrow. Time 15 sec.

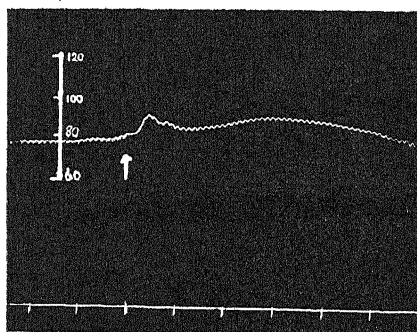


Fig. 2. 0.3 mg. adrenaline at arrow. Time 15 sec.

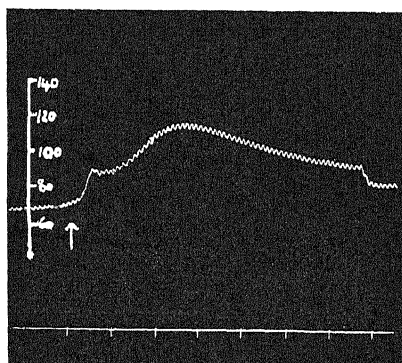


Fig. 3. 0.3 mg. adrenaline at arrow. Time 15 sec.

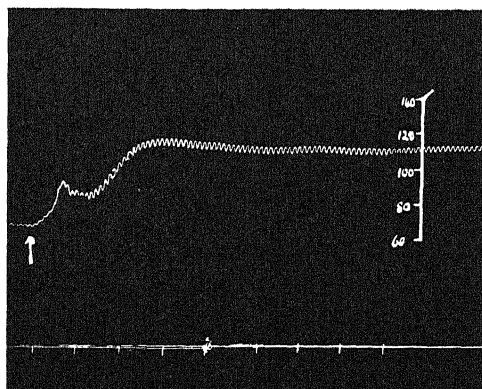


Fig. 4. 0.3 mg. adrenaline. Time 15 sec.

onward the pupils were constricted to a maximum, so that it appears that there may be a certain degree of selectivity in the sympathetic paralysis induced by ergotamine. From the 80th to the 140th min., corresponding to the period of complete inhibition of the pressor effect of adrenaline, there occurred the fall in blood sugar previously referred to. The blood lactate during the same period remained completely unchanged. The rise in lactate from the 140th to the 200th min. we attribute to the demonstrably diminished effectiveness of the ergotamine, but as the blood sugar remained unchanged during this time, even with the very large doses of adrenaline used, it is possible that there is some dissociation of the two mechanisms.

In the next experiment a larger dose of ergotamine was used.

TABLE III.

Cat. 3.25 kg. 24 hours without food. Amytal 300 mg. intraperitoneally. Anaesthetic in 10 min. Cannulae in left carotid and left femoral arteries. Blood-pressure recorded from the latter.

Time min.	Blood		Rectal temp.	Remarks
	Sugar mg./100 c.c.	Lactate mg./100 c.c.		
0	143	100	39.0	High lactate due to violent excitement.
20	Ergotamine methan sulphonate 9 mg. intravenously.			
25	Respiration failed. Artificial respiration put on.			
30	Pupils mere slits.			
40	139	—	—	
60	135	54	39.0	
68	Adrenaline 1 mg. subcutaneously.			
105	111	33		Note fall in lactate and sugar.
135	60	75		Mishap to pump. Anoxæmic rise in lactate.
183	Tracing of blood-pressure commenced. Three doses of adrenaline 0.2 mg. at intervals of about 4 min. Figs. 5 and 6.			
188	81	54	39.0	
225	87	56	39.0	

In this experiment the total adrenaline injected was 0.6 mg. intravenously and 1 mg. subcutaneously, the total time of action being $2\frac{1}{2}$ hours. Unfortunately the animal became very excited after the injection of the amytal as a result of an explosive noise in the laboratory, so that a very high initial lactate was found. The injection of the ergotamine was followed in 40 min. by a fall in blood lactate from 100 to 54 mg./100 c.c. As before, the injection of adrenaline was followed by a fall in blood sugar, in this case to 60 mg./100 c.c. in 67 min. At this point the respiratory pump gave some trouble, and at once there occurred a large rise in blood lactate, which suggests that asphyxial release of lactic acid is not inhibited by ergotamine.

The subsequent injection of three doses of 0.2 mg. adrenaline led in each case to atypical pressor responses. As before, each injection produced a sharp rise in blood-pressure followed by a fall and then a prolonged rise or a rise and a plateau (Figs. 5 and 6). During this period the blood sugar rose by some 20 mg./100 c.c., whilst the lactate remained at the same level as before the injection of the initial dose of adrenaline 165 min. before. Thus no significant change in blood lactate occurred as a result of these injections of adrenaline.

In the next experiment the anæsthetic was urethane.

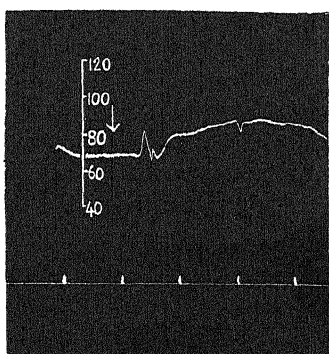


Fig. 5.

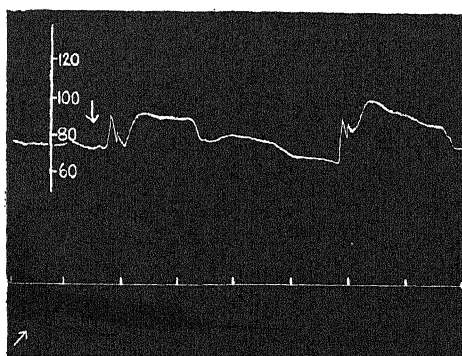


Fig. 6.

Figs. 5 and 6. 0.2 mg. adrenaline at arrow. Time in minutes.

TABLE IV.

Cat. 3.8 kg. 24 hours without food. Urethane 7 g. subcutaneously. Artificial respiration from the beginning of the experiment. Cannula for blood sampling in right carotid.

Time min.	Blood		Rectal temp.	Remarks
	Sugar mg./100 c.c.	Lactate mg./100 c.c.		
0	330	25	37.0	
30	296	22	37.5	
35	Ergotamine methan sulphonate 5 mg. intravenously.			
50	Pupils: R. 2 mm., L. 0 mm.			

Urethane raises the blood sugar to very high levels, but we have found that the blood lactate is not raised in cats if there is no anoxæmia. This was well seen in this experiment when the blood sugar was 300 mg./100 c.c. and the lactate was 25 mg./100 c.c. The ergotamine had no influence on the blood sugar, but the lactate was diminished to a steady level of 11 mg./100 c.c. The injection of adrenaline in large doses did not produce any significant effect on the lactate in 113 min.

DISCUSSION.

Although the exact mechanism by which adrenaline accelerates hepatic glycogenolysis is not known, the general view is that the sympathetic fibres to this organ exert a kind of motor control over this process. This is corroborated by the fact, among others, that ergotamine in addition to paralysing the vaso-constrictors also inhibits adrenaline hyperglycæmia. Adrenaline hyperglycæmia is accompanied by an increase in blood lactate, the height reached in both cases depending on such factors as peripheral oxidation, excretion by the kidneys and, in the case of lactate, resynthesis to liver glycogen. Other agents than adrenaline also produce hyperglycæmia and increased blood lactate, *e.g.* asphyxia, anoxæmia and certain anæsthetics. But high blood sugar is not necessarily associated with high blood lactate, *e.g.* in diabetes and in urethane anæsthesia. Nor must it be supposed that the adrenal glands are necessary for the production of high blood lactate. Thus in a cat from which the adrenal glands had been removed (the right gland $2\frac{1}{2}$ months before the left one) and which had then been starved for 2 days, it was found that the blood lactate, after anæsthetizing with urethane (2.5 g. in an animal of 2.7 kg.), was 80 mg./100 c.c. The injection of adrenaline in this animal rapidly raised the blood lactate to 140 mg./100 c.c. That urethane itself does not raise the blood lactate was again shown in a cat of 2.75 kg. anæsthetized with 4 g. urethane subcutaneously. Estimation of blood lactate at frequent intervals during 300 min. gave the following values: 26, 22, 19, 21, 18, 17, 20, 16, 21 mg./100 c.c.

The liberation of lactic acid from muscle glycogen is not solely dependent on adrenaline, so that it would be difficult to demonstrate conclusively a dual action of this hormone on the liver and muscle glycogen. To suggest that the mechanisms are different fundamentally is to suppose that adrenaline may act otherwise than by means of the sympathetic. Our evidence on the anæsthetized animal does not lead us

to make any such assumption. The distinction lies not in the type of action but in the response of which the tissue is capable.

CONCLUSION.

In the cat anæsthetized with amytal or urethane, ergotamine inhibits both the hyperglycæmia and the increase in blood lactate which normally follow the injection of adrenaline.

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THE EFFECT OF ACTIVITY ON THE FORM OF THE MUSCLE TWITCH.

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It has been found by various workers [Hartree and Hill, 1921; Bronk, 1930; Bozler, 1931; Feng, 1931] that in isolated muscle an increase occurs, during a prolonged contraction, in the economy with which the contraction is maintained. Bozler found in the retractor of the pharynx of the snail that with one shock every 3 sec. an incomplete tetanus was at first obtained, gradually developing into a smooth contraction. The rate of heat production gradually diminished while the tension remained constant. Feng [1931] has related this increase of economy to a progressive slowing of the muscle. Bronk [1933] has given a striking demonstration of the same phenomenon in crab's muscle.

Hill [1931], during the course of experiments with the frog's gastrocnemius, observed during a series of twitches a very rapid "spreading-out" of the contraction. It was more noticeable in large Hungarian *R. esculenta* than in Dutch *R. esculenta* or English *R. temporaria*. In his paper (Fig. 7, p. 293) are shown series of single twitches at 3 and 2½ sec. intervals. The "spreading-out" of the contraction is immediately obvious, the sixth twitch of a series lasting almost twice as long as the first twitch. In tetani the summation was at first incomplete, but became complete as stimulation continued. It was also found that the slowing occurred earlier in the later series of twitches and that recovery was almost complete in 30 sec. This recovery was so quick that it had obviously nothing to do with the ordinary oxidative recovery process, especially since it occurred equally in large gastrocnemii so thick that oxygen could not possibly have penetrated into the interior.

The experiments described in this paper represent an attempt to investigate further this "spreading-out" of the contraction in twitches. Since the rapid recovery observed could not be associated with oxidation, the question naturally arose: could it be due to the delayed lactic acid

formation which is known to be associated with the restoration of phosphagen [see *e.g.* Lundsgaard, 1930]? The question could be answered by seeing whether the "spreading-out" occurred, and whether, if it did, it was followed by equally rapid recovery, in a series of twitches in muscles poisoned with iodoacetate. It was desirable also to know whether it came on as quickly in muscles which were entirely rested and normally supplied with blood through the circulation.

A. EXPERIMENTS ON ISOLATED NORMAL GASTROCNEMIUM.

Preparations from large Hungarian *R. esculenta* were used in the first experiments. The muscle was left attached to part of the femur, which was held in an ebonite clamp. This was fitted to a frame of the type described by Hill [1931]. One end of a thin, straight wire was tied to the tendon and the other end attached to an isometric lever. The nerve was stimulated through a pair of silver electrodes: the stimuli used were provided by condenser discharges, single shocks being given by hand at the required intervals with a Morse key. The muscle was surrounded with Ringer's solution (buffered to pH 7.2 by phosphate solution 10 mg. P per 100 g.) for about 30 min. before an experiment, the solution being stirred by oxygen or nitrogen.

In the earlier experiments the following procedure was adopted. A series of ten stimuli was given at intervals of $\frac{1}{2}$ to 2 sec. and one stimulus each at 30, 45 and 60 sec. after the last of the series. The object of the three later shocks was to determine the interval necessary for the recovery or partial recovery of the muscle. The mechanical response was recorded on a rapidly moving smoked drum driven by a constant speed motor.

Experiments performed in this way confirmed the observations of Hill [1931]. The first twitch of a series was invariably less prolonged than the succeeding ones, "spreading-out" of the contraction being obvious in the third or fourth twitch. Recovery was generally complete in 60 sec., or at least as complete as it would be finally. Successive series showed increasing "spread." The effects were observed in nitrogen as well as in oxygen.

B. EXPERIMENTS ON ISOLATED POISONED GASTROCNEMIUM.

In order to determine whether lactic acid formation was connected with this phenomenon experiments were made on muscles poisoned with mono-iodoacetic acid (I.A.A.). Since it was impossible to poison a gastrocnemius effectively by soaking in Ringer's solution containing the acid, it was necessary to inject the poison into the frog. The following

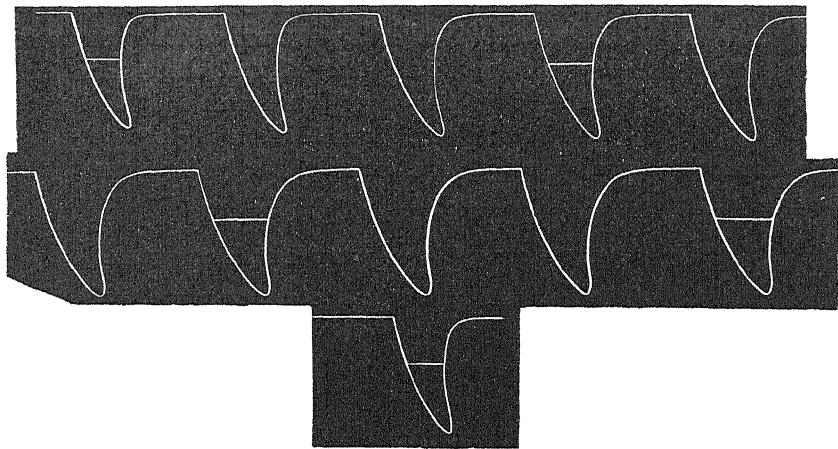


Fig. 1. First two rows: first series of ten twitches at 1 sec. intervals of fresh isolated gastrocnemius of Hungarian *R. esculenta*. The tenth twitch lasted about twice as long as the first. (The duration of each twitch is measured at 40 p.c. of its height.) Note that the response increased slightly in size during the series. Third row: single twitch taken 60 sec. after the last of the series. "Recovery" was almost complete, both in width and height.

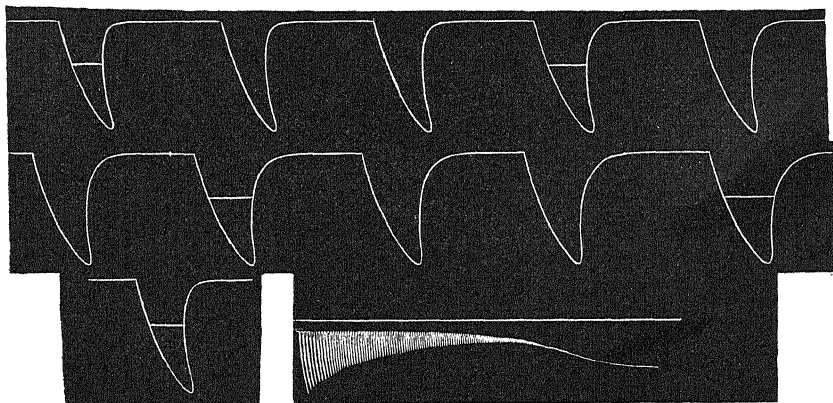


Fig. 2. Series of twitches, similar to Fig. 1, by the opposite gastrocnemius of the same frog, but poisoned with iodoacetate. The "spreading-out" was almost identical with that observed with the normal muscle, and "recovery" was again complete in 60 sec. The adequacy of the poisoning is shown by the typical I.A.A. contracture setting in after sixty to eighty twitches (drum slowed).

method was adopted. The frog's brain was pithed and the skin cut at the back of the leg, avoiding damage to the dorsal lymph sac, the sciatic nerves were exposed and carefully separated from adjoining arteries. Fine silk threads were then tied round the nerves about 2 cm. from the junction with the muscles and the nerves cut through above this point. Care was taken to avoid loss of blood. The skin was replaced and the frog injected in the dorsal lymph sac with iodoacetic acid (in the form of the sodium salt), 2 c.c. of a 2 p.c. solution per 50 g. of frog being given. When the forelimbs became rigid (in $\frac{3}{4}$ to $1\frac{1}{4}$ hours) the frog was laid on ice and the muscles with nerves dissected and placed in ice-cold Ringer. Generally speaking, the whole frog, with the exception of the gastrocnemii, was rigid before dissection was complete.

The muscles were mounted in frames and placed in Ringer's solution at temperatures varying from 7.5° C. to 18° C. The Ringer's solution was stirred by nitrogen. After 15 min. the solution was withdrawn and the experiments made in nitrogen.

The same "spreading-out" was observed in these poisoned muscles, though only three or four series of ten twitches were possible before contracture began. The contracture, which was large in every case, proved that the poisoning was adequate.

It seemed desirable for comparison to use a poisoned and a normal muscle from the same frog. The brain having been destroyed and the nerves cut, the frog was left for 2 hours with the circulation intact to recover from previous activity. (Recovery by soaking in oxygenated Ringer's solution would take too long in the case of a muscle as thick as the gastrocnemius of a Hungarian frog.) A ligature was then tied round the upper part of one thigh and this leg cut off. The frog was then injected with i.a.a. The muscle from the normal leg was used for experiment immediately and the poisoned muscle used when the frog had become rigid.

The "spreading-out" of the contraction appeared to be the same in both normal and poisoned muscles, and "recovery" also occurred in both.

These results, as well as those described in the following sections, were found in a large series of experiments without exception.

C. NORMAL AND POISONED GASTROCNEMII *IN SITU*.

In these experiments the conditions for normal and poisoned muscles were identical. The brain was destroyed; the nerves were tied and cut as usual and the skin around each Achilles tendon was removed. A thread

was tied round the tendon and the latter cut just below it, avoiding injury to the blood vessels. The frog was then pinned down on a board, the thread from one tendon connected to one arm of a crank, and the other arm of the crank (at 90°) attached by a thin wire to an isometric lever above. The crank was light, frictionless, and adjustable in a horizontal direction: the isometric lever was attached to a heavy stand adjustable vertically.

The frog was left in the position described for 2 hours. Since the circulation was not hindered in any way, both muscles recovered in this time from activity during dissection. When required for stimulation the nerves were raised by threads and laid across a pair of silver electrodes fixed to the board; an initial tension of about 10 g. was placed on the muscles by raising the isometric lever.

When the experiment on the normal leg was complete the tendon of the other leg was attached to the crank, the frog was injected with the appropriate amount of I.A.A. and allowed to remain until the forelimbs became rigid. The muscle was then stimulated and responses and contraction recorded.

It was noticed in these experiments that the "spreading-out" of the contraction began much later than in the isolated muscles. In the latter, 50 to 100 p.c. "spread" was observed in the tenth twitch, but fifty to seventy twitches were necessary to produce the same "spreading-out" in muscles *in situ*. In the tenth twitch no "spread" was noticeable.

A highly rested condition is clearly unfavourable to the appearance of the phenomenon in question.

D. THE EFFECT OF CIRCULATION IN NORMAL GASTROCNEMII.

In order to determine whether the circulation continuing during stimulation had any effect on retarding the "spread" in muscles *in situ*, the following experiment was made. The frog was prepared as described above, and a record was obtained from a normal muscle with circulation intact. Immediately before stimulation of the other muscle a ligature was tightly tied round the upper part of the leg in order to stop the circulation.

The muscles exhibited almost identical responses. The normal muscle recovered in about 20 min. from the fatigue produced by 200 twitches, while, as expected, the muscle without circulation showed no recovery. Apparently the absence of circulation during such rapid stimulation does not affect the "spreading-out."

E. EXPERIMENTS ON ISOLATED SARTORIUS MUSCLES.

Isolated sartorius muscles from Hungarian *R. esculenta* exhibit the same "spreading-out" of the contraction as the gastrocnemii. The muscles were soaked in oxygenated Ringer's solution for at least 2 hours to avoid reversible inexcitability [Dulière and Horton, 1929]. Nitrogen was then passed through the Ringer for 30 min., the Ringer was withdrawn and stimulation (direct) carried out in nitrogen.

Poisoning with I.A.A. by soaking in Ringer's solution containing 1 part in 12,500 of that substance had no effect on the "spreading-out." The muscles exhibited a typical poison contracture after about 100 twitches.

The effect of varying the pH of the Ringer surrounding the muscle was examined. After 2 hours in oxygenated Ringer the muscle was stimulated in nitrogen and the responses recorded. The Ringer was then replaced and oxygen passed through it for 30 min. The oxygen was then replaced by a mixture of 5 p.c. CO₂ and 95 p.c. nitrogen, which was allowed to bubble through the Ringer for about 1 hour. The Ringer was then removed and the muscle stimulated in the atmosphere of CO₂ in nitrogen. The "spreading-out" obtained after this treatment was identical with that obtained in oxygen. The Ringer's solution at the end of the experiment had a pH of 5.6.

An experiment was made on the sartorius muscle first under normal conditions and then after soaking for 1½ hours in calcium-free Ringer's solution. The "spreading-out" and recovery remained unaltered.

A pair of sartorius muscles from a curarized frog also showed the same phenomenon.

Sartorius muscles from Dutch *R. esculenta* and English *R. temporaria* behaved in a similar manner to those of Hungarian frogs but to a less degree.

F. TEMPERATURE AND FATIGUE.

Alteration of the temperature appeared to have no effect on the "spreading-out" of the contraction; the "spread" was noticeable as early as the tenth twitch and more noticeable in later twitches. No fatigue was seen even after 50 or 100 twitches—indeed the response was generally greater than at the beginning.

DISCUSSION.

Contrary to expectation, the recovery which sets in rapidly after a few twitches and causes the return of the normal form of contraction within a minute has nothing to do with the delayed lactic acid formation

by which phosphagen is restored. Quantitatively the "spreading-out" and recovery are very striking phenomena, at least in Hungarian frogs, and at present one can suggest no chemical reaction corresponding to either of them. Complete previous oxidative recovery is obviously not favourable to the rapid onset of the "spreading-out," but it does not prevent it, only makes it necessary to give a larger number of twitches. It is not possible at present to do more than describe the phenomenon. Its relation to chemical processes occurring in muscle is not obvious.

SUMMARY.

During a series of muscle twitches at intervals of a second or two a considerable "spreading-out" of the contraction occurs. "Recovery," which is independent of the presence of oxygen, is complete within a minute. The "spreading-out" is not connected with lactic acid formation, nor is the "recovery" associated with phosphagen restoration, since both occur unchanged in muscles adequately poisoned with iodoacetate. A completely resting condition (absence of stimulation with previous intact circulation) delays the "spreading-out," but its onset is not quickened by circulatory stoppage during the series. Sartorii showed the same phenomenon. "Fatigue" is not the cause of it, since usually the response increased during a series. Temperature and *pH* have no effect on it. Its relation to known chemical changes in muscle is not obvious.

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THE RATE OF WATER ABSORPTION IN MAN AND THE RELATIONSHIP OF THE WATER LOAD IN TISSUES TO DIURESIS.

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INTRODUCTION.

WHEN water is taken by mouth the rate of absorption cannot be assumed [Heller and Smirk, 1932 *a, d, e*]. For the interpretation of the resulting diuresis it is therefore desirable to have some method of estimating the rate of absorption. It is known [Tashiro, 1926; Baer, 1927; Glass, 1928; Heller and Smirk, 1932 *a, c*] that the water absorbed passes mainly to the muscles and skin. Hence there should be a decrease in the weight of the abdomen and an increase in the weight of the limbs during absorption: and the weight of the limbs should decrease as the excess of water is excreted. The following method was devised for the purpose of measuring such weight changes in man and thus deducing the rate of absorption.

EXPERIMENTAL PROCEDURE.

Abdomen weighing experiments.

The subject lies horizontally face downwards with the abdomen on the footplate (*D*) of the weighing machine (*B*) which is suitably padded (Fig. 1). The head, thorax and thighs rest on rigid but padded supports. In order that the weight of the buttocks may be partly counterbalanced by the weight of the legs, the lower two-thirds of the legs are without support and project over the padded block (*C*) which supports the thighs.

Weights placed in the scale pan (*E*) will balance whatever proportion of the abdominal weight is resting on the footplate, and if water is then taken by means of a rubber tube held in the mouth without any change in posture the weight of the abdomen is seen to increase rapidly as the

water is swallowed and then to diminish gradually as the water is absorbed.

Although even the movement of respiration is revealed by oscillations of the steelyard, it appears easy to maintain a steady posture, and, apart from respiration, spontaneous changes in the abdominal weight indicated are infrequent, and unless the subject has been unduly active beforehand this weight remains constant from a few moments after the position on the apparatus is assumed.

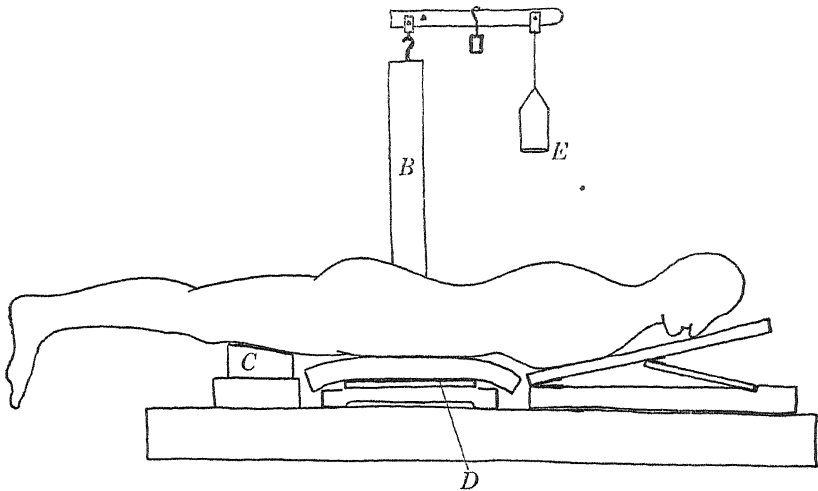


Fig. 1. Abdomen weighing experiment.

Leg weighing experiments.

The subject of this experiment lies on a couch. The head is slightly raised and the lumbar region is supported on a rounded elevation which combines comfort with stability of posture. The foot of the couch is a little higher than its head, and the padding must be of material such as horsehair which will allow the formation of a depression by the weight of the buttocks, but will not slip and make it difficult to immobilize the subject. The padding (a detachable hair mattress) is held in position by a block of wood *A* nailed to the wood at the bottom of the couch. A piece of board rests at one end upon the buttock just above the block *A*, and in the middle has a groove which is pivoted on the knife edge *C*. This knife edge is fixed to the footplate of the weighing machine *D*. The feet are tied together. The ischial tuberosities of the patient should be situated on the padding of the couch about 2 in. from the block of

wood *A*, and the position of the knife edge *C* should be near the bend of the knee. By placing suitable weights in the scale pan *E* the legs are balanced. It is necessary for the subject to remain in one posture, since movements even of the arms or head produce changes in the apparent weight of the legs. The movements of respiration are associated with oscillations of the balance pan. It is advisable for the subject to remain silent, as conversation is accompanied by irregularity and increased amplitude of the respiratory movements. The sensitivity of the system

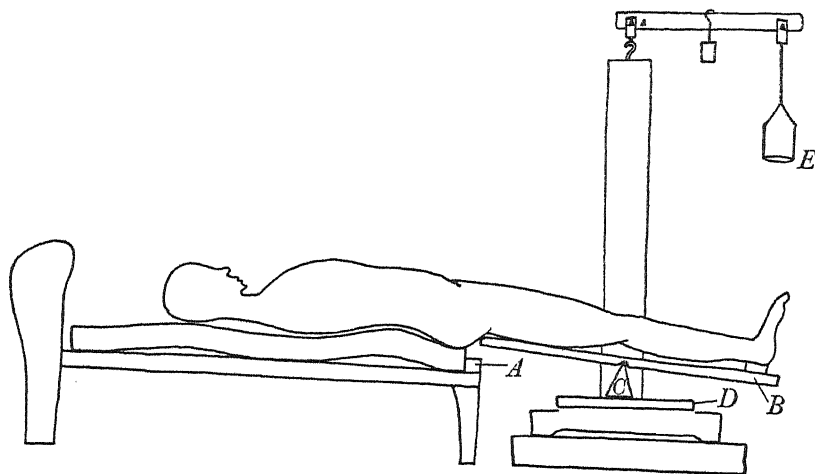


Fig. 2. Leg weighing experiment.

should be tested by placing a 10 g. weight by the feet. The subject should not be exposed to draughts or have the arms or legs exposed, and the experiment should be conducted in a warm room, since changes in limb volume and weight occur as a result of the vaso-motor changes induced by temperature regulation. It will be observed that for a few minutes after lying down the limb weight is somewhat variable, probably owing to vascular adjustments. At the end of 10 min. the limb weight is usually steady and observations of the basal weight are made. In experiments where it is necessary for the subject to be recumbent throughout, the water is taken from a rubber tube held in the mouth—otherwise he sits up while drinking. In either case the same weights are left in the balance pan and, when necessary, equilibrium is restored by means of the rider, and the sensitivity of the system is tested by a 10 g. weight. It is essential that the water should be at 37° C.

In a few experiments the apparatus was modified so as to allow the subject to occupy the sitting posture.

The bladder is emptied before starting, and the amount of urine is given in c.c. Samples of urine may be obtained during the experiment, avoiding as far as possible a change in posture, and the rider is then used to correct the disturbance of equilibrium due to altered posture.

RESULTS.

In Figs. 3 to 14 the vertical scale records the weight placed in the scale pan which counterpoises the weight of the abdomen or legs. 1 g. in the scale pan represents a change of 100 g. on the footplate. In the leg weighing experiments the balance is quite sensitive to 0.1 g. In the scale pan, and in the abdomen weighing experiments to 0.3 g. The abscissæ indicate the time relationships in the experiment.

Abdomen weighing experiments.

When the subject lies with the abdomen upon the footplate of the weighing machine and 1000 g. of warm water are drunk without change of posture the weight required to counterpoise the water is usually much more than 10 g. The reason for this is that the distension caused by the water raises the abdomen and throws a greater proportion of the body weight upon the footplate of the weighing machine.

Nevertheless, a 100 g. weight placed on the middle of the back above the footplate is counterbalanced by 1 g. on the scale pan and 1000 g. weight in this position is counterbalanced by 10 g. These weights placed over the thorax or legs cause relatively slight changes.

It does not follow that the absorption of each successive 200 c.c. of water will necessitate the removal of equal weights from the scale pan. It is usually found, however, that the additional weight in the scale pan which counterbalances the water consumed is roughly the same as the weight subsequently removed from the pan during water absorption.

Leg weighing experiments.

The weight in the scale pan again represents 1/100th part of the pressure upon the footplate of the weighing machine, which is a proportion of the actual leg weight and varies according to the posture. The pressure upon the footplate is generally increased by 200 or 300 g. after drinking 1000 g. of water.

Throughout any single experiment the weight needed to counterpoise the leg weight is a constant but unknown proportion of the actual leg

weight, so that if any changes in the apparent leg weight are expressed as a p.c. of the apparent leg weight this p.c. is also the p.c. change in the true leg weight. If the changes in leg weight represent proportionally equal changes in other parts of the body the excretion of say 0.5 p.c. of the body weight of urine should cause a 0.5 p.c. decrease in leg weight, and the absorption and non-excretion of say 1.5 p.c. of the body weight of water should cause approximately 1.5 p.c. increase in the leg weight. The average stable weight of the legs is called 0 p.c.

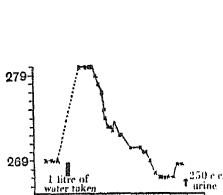


Fig. 3. Exp. 1 a. (S.)

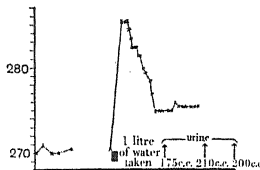


Fig. 4. Exp. 2 a. (S.)

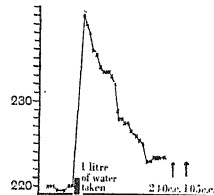


Fig. 5. Exp. 3 a. (N.)

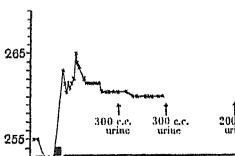


Fig. 6. Exp. 4 a. (S.)

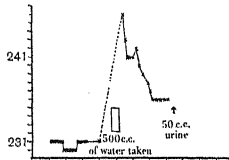


Fig. 7. Exp. 5 a. (S.)

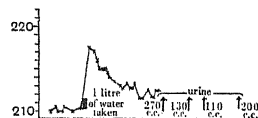


Fig. 8. Exp. 6 a. (N.)

Figs. 3-8. Changes in the weight of the abdomen during and after drinking water. Abscissa: Time of the experiment (10 minute intervals). Ordinate: Weight in g. needed to counterpoise the abdomen.

(a) *Control on the constancy of the abdominal weight when no water is given.*

It is quite clear from Exps. 2 a, 3 a, 4 a, 5 a, 6 a, that there is little difficulty in maintaining a steady posture and that the weight recorded, whatever relation it bears to the weight of the abdomen, is much less liable to extraneous variations than is the limb weight.

(b) *The decrease in abdominal weight during water absorption.*

In Exps. 1 a-6 a it will be seen (Figs. 3-8) that after drinking a litre of water the weight needed to counterpoise the abdomen is at once increased. After a delay, which varies from 0 to 10 min., the weight needed to counterpoise begins to decrease and continues to do so for a period which has varied in our experiments from 22 to 55 min. With the onset of diuresis the abdominal weight has sometimes risen again,

owing no doubt to the accumulation of urine in the bladder. There seems little doubt that the fall in weight is due to the absorption of water from the intestines and its distribution to peripheral parts of the body: the subsequent rise in weight to the withdrawal of water from the tissues and its return to the abdomen in the form of urine.

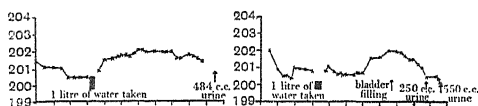


Fig. 9. Exp. 1. (N.) Fig. 10. Exp. 2. (M.)

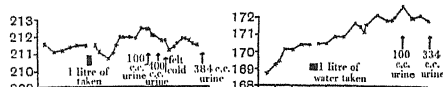


Fig. 11. Exp. 3. (S.) Fig. 12. Exp. 4. (N.)

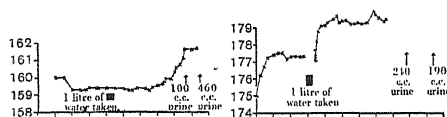


Fig. 13. Exp. 5. (N.) Fig. 14. Exp. 6. (N.)

Figs. 9–14. Changes in the weight of the legs after drinking water. Abscissa: Time of experiment (10 minute intervals). Ordinate: Weight in g. needed to counterpoise the legs.

(c) *Control on the constancy of the leg weight when no water is given.*

For several minutes after the subject has taken his place on the apparatus changes are observed in the leg weight, which are due presumably to adjustments in the volume of blood contained in the limb as a result of the altered posture. The direction of these changes is not constant. Often the limb weight increases (as in Exps. 4 and 6), sometimes it falls (Exps. 1 and 5), but after 10–15 min. the limb weight has usually reached a level which remains constant so long as the subject is undisturbed. This is particularly evident in Exps. 5 and 6. No spontaneous increase in leg weight occurs when the initial changes due to alteration of posture are complete.

(d) *Increase in leg weight after water drinking.*

It is sufficiently clear that an increase in the leg weight takes place after water drinking, which continues for as long as 25–55 min. after taking the water. It remains to be considered in Sections (e) and (f) if this increase in weight is caused mainly by the storage of water in the limbs.

- (e) *Evidence that a part of the observed increase in the weight of the legs may be the result of changes other than the storage of water.*

It is not suggested that the entire change recorded in the leg weight is an accurate representation of the storage of water. There is in Section (f) abundant evidence that the changes in leg weight after water drinking are mainly the result of water storage, but it is desirable to know something of the factors which prevent quantitative accuracy. Gross mechanical movements are readily detected by the subject, and cause no difficulty: they are accompanied as a rule by an erratic and immediate change in the apparent leg weight, which is distinct from the steadily progressing changes resulting, as I believe, from water storage. These gross changes should be corrected by adjustments of the rider (see Fig. 2) so that the weight which was in the scale pan before the movement took place once more will balance the weight of the leg. But smaller changes in posture may doubtless take place without the knowledge of the subject. These will be recorded and constitute a source of experimental error. A subject who has remained in one posture for over an hour may be troubled by muscular twitches of a sufficient magnitude to change the apparent weight. Where this has happened, either the experiment has been terminated or the subject allowed to rest on his side for a few minutes before resuming the original posture. Adjustments are then made with the rider.

Changes in the distribution of blood about the body are part of the mechanism for temperature regulation. An account of some of the vasomotor responses to a need for thermo-regulation has been published by Pickering [1932]. No accurate measurements of changes in the limb weight resulting from thermo-regulation have been made in these experiments, but it has been noticed on several occasions that the immersion of one hand in cold water was associated with appreciable diminution in the leg weight. The counterpart to this appears in a paper of Gibbon and Landis [1932] who have observed a vaso-dilation in the legs following the application of heat to an arm.

In Exp. 9 the temperature of the water when taken was well above 37° C. Soon the subject felt flushed and noticed a moderate degree of sweating which lasted for 2 min. The leg weight meanwhile increased rapidly to a peak—an increase in the leg weight of 3.1 p.c. having taken place within 7 min. The weight then fell rapidly by 1 p.c. of the limb weight in 2 min. and then rose more gradually, the total increase being 3.8 p.c. of the body weight. Since only 1.3 p.c. of the body weight of

water had been given it is clear that this rapid increase in the weight of the limbs must in part represent something other than the storage of water. It is probable that the gradual increase, and the gradual fall corresponding to the increased urine formation, were the result of water storage and excretion, but superimposed are two peaks which are the result of vaso-motor disturbance.

In Exp. 3 the water taken was cooler than 37° C. and an initial fall in leg weight was recorded.

Without entering into further details it is evidently desirable that the precautions suggested in the experimental procedure should be followed strictly, and all possible care taken to minimize errors.

(f) Evidence that the observed changes in the weight of the legs and abdomen are mainly the result of water storage and excretion.

In Section (b) it has been shown that a record may be obtained which demonstrates changes in the weight of the abdomen corresponding to the absorption of water. When a litre of water has been consumed through a rubber tube without change of posture the weight required to counterpoise the abdomen increases and, after a short delay, diminishes again. The fall in weight has occupied periods of from 22 to 55 min. There seems every reason to believe that the fall of the abdominal weight is the result of water absorption, and an increase in the leg weight is to be expected from the storage of this absorbed water.

This increase in the weight of the legs is indeed observed, but it is necessary to present evidence that the change is actually the result of water storage.

If we take the changes in leg weight recorded towards the end of Exps. 1, 2 and 3, and for shorter periods in Exp. 4, it will be seen that as more urine is formed the leg weight decreases and, although the fall in leg weight does not tally precisely with the amounts of urine formed, it is of the same order. Thus in Exps. 1, 2 and 3 the leg weight has decreased 0.38, 0.90 and 0.40 p.c. in correspondence with a urine formation of 0.69, 0.79 and 0.57 p.c. body weight.

In Exp. 7 the subject had taken a large drink of water not long before the observations began. His basal rate of urine formation was therefore high and the weight of the legs was falling gradually. The second dose of water (715 c.c.) caused a rapid increase in the rate of urine formation, so that 640 c.c. of urine were excreted within 40 min. The leg weight did not alter appreciably, as the accumulation of water in the body was prevented by the previous establishment of diuresis.

In Exps. 2 and 5 the subjects had lunched 1 hour previously, and during the experiment drank their water lukewarm in the horizontal posture. For periods of about 20 min. both subjects complained of a marked sensation of gastric distension which passed off just before the leg weight began to increase. Previous to this the leg weight had been constant. There seems every reason to believe that a large part of the water was retained in the stomach, and delay in the increase of leg weight was due to non-absorption of the water. This suggestion is supported by the fact that there was a rather longer delay before the onset of diuresis.

If we examine the degree of increase in the water content of the legs at the time when the leg weight has reached its maximum but before urine formation has increased greatly, the actual figures are 0.85, 0.75, 0.65, 1.45, 1.35, 1.35, 1.70 p.c., averaging 1.15 p.c. leg weight. If the leg represents the rest of the body the increase in total body weight is also 1.15 p.c. The quantity of water given (as a p.c. of body weight) is about 1.3 p.c., and making due allowance for experimental errors this is a surprising approximation.

These results, compared with those recorded in the last of the subsequent papers where diuresis was prevented by pituitrin, show clearly that the addition of water to the body causes an increase and removal of water a decrease in leg weight.

(g) *The rate of water absorption.*

Section (f) would justify the assumption that the changes in the weight required to counterpoise the legs and the abdomen after water drinking are mainly the result of water absorption. Yet, as mechanical and vaso-motor changes influence the magnitude of the deviations, one cannot deduce accurately the amount of water absorbed from the degree of change in the weight of the leg or abdomen. But so long as appropriate changes are taking place in these weights, it is likely that absorption is still in progress, and when a steady state has been attained, that the process of absorption is over. Therefore, irrespective of the magnitude of the changes, the water given is assumed to be absorbed when the weight of the legs or abdomen has reached a steady state. If, however, the rate of urine formation has increased to a degree which equals the rate of absorption a correction must be applied, as in this instance there will be no further increase in the leg or decrease in the abdomen weight, and completion of the process of absorption may appear to be earlier than it actually is. It will be seen however (Section (h)) that the error from this cause is usually small, and it may be eliminated

entirely if the increased urine formation is prevented by pituitary hormone (see the last of the subsequent papers). It is, therefore, believed that, when due correction has been made for the amount of urine formed, the completion of water absorption may be determined either by the

TABLE I. The rates of water absorption in man determined by counterpoising the abdomen.

Exp. No.	Factors which may have influenced the rate of water absorption	Time expended in the absorption of 1 litre of water (min.)
1 a. (S.)	Water taken 2.5 hours after lunch. Temperature of water exactly 37° C. Sensation of gastric distension for 8 min.	55
2 a. (S.)	Water taken 2.5 hours after lunch. Temperature of water exactly 37° C. Sensation of gastric distension for 2 min.	27
3 a. (N.)	Water taken 3 hours after lunch. Temperature of water exactly 37° C.	40
4 a. (S.)	Water taken 1.5 hours after breakfast of egg in milk. Temperature of water exactly 37° C.	Doubtful
5 a. (S.)	Water taken 2.5 hours after lunch. Temperature of water exactly 37° C. No sensation of gastric distension. Water taken in the standing posture	22 (only 500 c.c. water taken)
6 a. (N.)	Water taken 3 hours after breakfast. Temperature of water exactly 37° C.	35

TABLE II. The rates of water absorption in man determined by counterpoising the legs.

Exp. No.	Factors which may have influenced the rate of water absorption	Time expended in the absorption of 1 litre of water (min.)
1. (N.)	Water taken 3 hours after lunch. Temperature of water exactly 37° C. Horizontal posture	28
2. (M.)	Water taken 2.5 hours after lunch. Temperature of water exactly 37° C. Sitting posture. Sensation of gastric distension for 15 or 20 min. Delayed increase	45
3. (S.)	Water taken 3 hours after breakfast. Temperature of water below 37° C. Horizontal posture	30
4. (N.)	Water taken 1.5 hours after lunch. Temperature of water about 37° C. Horizontal posture	55
5. (N.)	Water taken 2 hours after lunch. Temperature of water about 37° C. Horizontal posture. Sensation of gastric distension for about 20 min. Delayed increase	45
6. (N.)	Water taken 2.5 hours after lunch. Temperature of water exactly 37° C. Sitting posture. No sensation of gastric distension	20-25
7. (R.)	Water taken 1.5 hours after lunch. Temperature of water about 37° C. Horizontal posture	About 50
8. (S.)	Water taken 2.5 hours after breakfast. Temperature of water about 37° C. Horizontal posture	50
9. (S.)	Water taken 2.5 hours after lunch. Temperature of water well above 37° C. Sitting posture. No sensation of gastric distension	35

cessation of decrease in the weight of the abdomen or by the cessation of increase in the weight of the legs.

The above tables summarize the rates of water absorption so determined, and indicate some of the factors which probably influence this rate.

It will be seen that the time taken to absorb a litre of water at 37° C. has varied between 25 and 55 min. and that in the single experiment where only 500 c.c. of water were taken absorption was completed in 22 min. In the two experiments where water was taken 1.5 hours after lunch the times of absorption were 55 and 50 min. In the four experiments where water was not taken until 3 hours after a meal the times of absorption have been 30, 28, 40 and 35 min. While there is no accurate relationship water appears to be absorbed more rapidly after a 3 hours' fast, and this is probably related to the rate of emptying of the stomach.

If we refer to the instances where the rate of water absorption has been determined by weighing the legs, it is usually observed that there is a period of delay before the weight begins to increase at all. It is possible that this delay is occasioned by the holding up of a large part of the water in the stomach. This is supported by the subjective sensations of the victim. In most experiments the subjects have been asked to observe the duration of a sense of gastric distension. Usually gastric discomfort passes off shortly before the leg weight increases. If water absorption is determined by counterpoising the abdomen there is seemingly a shorter period of delay before the weight of the abdomen begins to decrease. The duration of the discomfort from gastric distension is less in the prone posture, but more marked while it lasts. In the prone posture emptying of the stomach is probably more rapid as a result of the increased intra gastric tension.

It has been mentioned that, if the rate of urine formation had increased much before absorption was ended, there would be an error in our calculation of the absorption rate. Estimated by counterpoising the abdomen there would be a deceptive period when the fall in weight occasioned by absorption is balanced by an increase due to accumulation of urine in the bladder. When the leg weight is counterpoised the increase due to storage is balanced by the withdrawal of water for excretion by the kidneys. In actual practice, however, the error is not great.

When diuresis is prevented by pituitary hormone this error is eliminated and it will be seen in the last of the subsequent papers that the time expended in attaining the maximum leg weight is not increased thereby. The time expended in water absorption appears the same in the

subjects receiving 0.1 c.c. pitressin as in the experiments where diuresis was allowed to proceed normally.

(h) *The relationship of the water load to diuresis.*

It will be clear from the preceding sections that the absorption of a litre of water in man usually nears completion before diuresis starts. One may refer, however, to the actual amounts of urine formed in various experiments. When the completion of water absorption was determined by counterpoising the legs the amounts of urine formed when water absorption was ended averaged something under 230 c.c. and in the abdomen weighing experiments under 200 c.c.

Now it is evident from the examination of many diuresis curves in man that where 1000 c.c. of water have been given the maximum rate of urine formation is reached when about half of the urine to be excreted has been formed, and that when absorption is complete the amounts of urine already formed are much less than half of the urine to be excreted. From the amounts of urine recorded on Figs. 3-14 it can be calculated that the rate of urine formation continues to increase after the completion of absorption. We may conclude from this that absorption is complete before the maximum diuresis is attained, and it follows that the maximum load of absorbed but as yet unexcreted water in tissues must be present some time before the maximum rate of urine formation is attained.

DISCUSSION.

It will be clear from the foregoing results that, when a man drinks water, an increase in weight can be detected later in situations outside the alimentary canal and at times which correspond to a decrease in the weight of the abdomen. Thus for 25-55 min. after drinking water the limb weight has continued to increase, but after this time (which varies in individual subjects) there is no further increase in the weight of the legs. Where pituitrin has been given (see the last of the two subsequent papers), and diuresis thereby prevented, no further increase in leg weight takes place after 25-55 min., and as the water is but slowly excreted this increased leg weight is maintained. The degrees of increase in weight have been 0.85, 0.75, 0.65, 1.45, 1.35, 1.35, 1.70 p.c., averaging 1.15 p.c. of leg weight, the amount of water given being approximately 1.3 p.c. of body weight. The order of increase is in general roughly what would be expected from the amount of water given. An accurate correspondence between the p.c. increase in limb weight and the p.c. body weight of water given should not be anticipated, since vaso-motor changes and undetected movements

play their part in determining the final weight recorded. Nevertheless, at the time when either the weight of the legs ceases to increase or the weight of the abdomen to decrease, it is very likely that water absorption is complete, so that knowing the volume of water given, the approximate of absorption can be ascertained.

If diuresis starts before absorption is complete we must consider the volume of urine excreted. In Exp. 7 where 715 c.c. of water were taken, the rate of urine formation was considerable at the outset owing to previous drinking of water and the weight of the legs was not greatly changed. In this experiment it will be seen that 640 c.c. of urine were excreted in 40 min., and since the limb weight remained unaltered we must consider that roughly 640 c.c. of water have also been absorbed in these 40 min. to replace the water lost as urine. In the experiments where it has been possible to continue observations for a sufficient time the leg weight may be observed to fall about the time when the subject becomes aware of a full bladder. At this time there is sometimes a slight increase in the weight required to counterpoise the abdomen, probably due to the accumulation of urine in the bladder.

In a number of experiments the water was taken about 1.5 hours after the mid-day meal. A sensation of distension was produced which lasted some 20 min., during which time the weight of the legs remained steady. Shortly after this the limb weight began to increase in the normal manner. It is not unlikely that a proportion of the water given was then held back in the stomach, and the onset of absorption processes delayed.

Parallelism is evident between known changes in the water content of the body and changes in the weight of the legs and the abdomen. If the suggested precautions are observed this decrease in abdomen weight and increase in leg weight is determined mainly by the absorption and storage of water in the tissues. It must be emphasized that vaso-motor changes produced in many ways, but chiefly by alterations in room temperature or by drinking too cold or too hot water, may also cause appreciable alterations in limb weight.

The principle of weighing separately the arms, legs, abdomen and thorax has been used previously by Müller [1905] and by Mosso [1884] for determining gross changes in the disposal of blood about the body. The alterations in leg weight described by Müller are similar to the variations I have also obtained as a result of vaso-motor reactions induced by cooling other parts of the body and do not resemble the changes due to water absorption.

SUMMARY.

1. An apparatus has been described which records changes in the weight of the abdomen after water drinking.

2. An increase in the weight of the abdomen is observed at once followed by a gradual decrease. The fall in weight is produced by the absorption of water and ends when all the water is absorbed.

3. This observation may be used to determine the absorption time for water which has been 22-55 min. in six experiments on two subjects in which 1 litre of warm water was consumed.

4. An apparatus has been described which measures an increase in the weight of the legs after water drinking in man and a decrease as this water is excreted by the kidneys.

5. The increase in leg weight has usually attained its maximum value 25-55 min. after giving water, which represents in nine experiments on four subjects the times expended in the absorption of 1 litre of water taken at 37° C.

6. It is thought that this increased weight results mainly from the storage of water which has been absorbed from the alimentary canal. The average degree of increase (1.15 p.c. of leg weight) is a probable one in consideration of the dose of water administered (1.3 p.c. of body weight).

7. The rate of water absorption is such that most of the water has been absorbed before there is any great increase in the rate of urine formation; so that the maximum load of absorbed but as yet unexcreted water is attained before the maximum diuresis.

I wish to thank Dr W. H. Newton for a helpful suggestion.

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THE EFFECT OF WATER DRINKING ON THE BLOOD
COMPOSITION OF HUMAN SUBJECTS
IN RELATION TO DIURESIS.

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INTRODUCTION.

THE results of experiments on animals [Smirk, 1932; Heller and Smirk, 1932 *a, b*] and on the rate of water absorption in man (see the previous paper) taken together afford evidence as to the relation between water absorption, water storage, blood composition and diuresis in man. The problem, however, of the relation between the water content of blood and diuresis is complex, and its experimental investigation is difficult.

In experiments on water diuresis the study of blood dilution is usually concerned with:

- (1) How much of the additional water given is stored in the blood?
- (2) Does any change take place in the water content of the blood which would be likely to affect the kidney? (on the supposition that the kidney may be directly influenced by small changes in the water content of blood).

These problems are different, and the determination of the percentage by weight of water in blood is no certain measure of the amount of ingested water retained in the circulation.

For example, if a change takes place in the proportion of corpuscles and plasma as a result of splenic contractions [Barcroft, Harris, Orahovats, Weiss, 1925], posture [Thompson, Thompson and Dailey, 1928], or local passive congestion [Smirk, 1928], then, since the proportion of solid is greater in corpuscles than in plasma, the percentage water content of a whole blood sample will decrease as the percentage by volume of the corpuscles increases. Again, if for any reason the amount of transudation from blood into lymph spaces is increased, then the water

content of the blood will depend upon the percentage of solids in the transudate which has been lost. In other words, an increase in the water content of blood, as determined by the percentage of solid residue after drying, may merely represent an increase in the proportion of plasma or a loss of one of the solid constituents of plasma such as the salt or protein. Moreover, two solutions each containing 95 p.c. of water and in one case 5 p.c. of protein and in the other 5 p.c. of NaCl are physically and physiologically very different solutions.

To give "blood dilution" a precise meaning it is, therefore, necessary to state the substance used as an index of dilution and the nature of the diluent.

THE SIGNIFICANCE OF VARIOUS INDICES OF BLOOD DILUTION.

The plasma chloride.

Dilution of the plasma chloride is for most practical purposes an approximate index of increase in the proportion of water molecules to total molecules in a protein-free filtrate of plasma. If the protein of plasma is also diluted analysis of the plasma chloride would give a slight underestimate of the increased water content of protein-free filtrate. Changes in the CO₂ content of blood will alter the distribution of Cl between plasma and corpuscles.

The whole blood chloride.

The whole blood chloride is uninfluenced by the CO₂ content of blood, but, since the plasma contains twice as much Cl as the corpuscles, it depends as much upon variations in the proportion of plasma and corpuscles as upon changes in the chlorine content of plasma. If blood is diluted with saline of Cl concentration less than plasma (0.6 p.c.) and greater than the whole blood chlorine (0.45 p.c.) then the plasma chloride falls and the whole blood chloride rises. A fall in whole blood chloride is, therefore, not necessarily an index of a diminished chlorine content in protein-free filtrate of plasma.

The conductivity of plasma.

The conductivity of whole blood depends largely upon the relative volume of plasma and red blood cells, but the conductivity of plasma depends for the most part upon the amount of dissociated electrolytes. The effect of plasma protein *per se* upon the electrical conductivity is slight in view of the large molecular weight and feeble dissociation.

A protein-free filtrate of plasma, however, will have a greater electrical conductivity than that of plasma owing to the presence of the same ionic concentration in a smaller volume of fluid.

The total osmotic pressure of blood.

This is the pressure required to prevent the diffusion of water into plasma, the water and plasma being separated by a membrane impermeable to all ions or molecules other than water. The pressure exerted by electrolytes will depend upon their molar concentration and the degree of electrolytic dissociation. The effect of non-electrolytes such as urea depends only upon their molar concentration. The total osmotic pressure may be ascertained indirectly by its effect upon the vapour pressure [Hill, 1930 a].

The protein content of plasma.

The protein content of plasma is probably the most reliable single index of general blood dilution and of the storage of water in blood. It is liable, however, to this error: in so far as additional water is stored as lymph the blood plasma will be deprived of the protein contained in that lymph. If the volume of transuded lymph is exactly replaced by the water entering from the gut, then a dilution of the plasma protein would result which would not be a true indication of the storage of additional water in blood.

Under certain circumstances this objection is not merely theoretical [Heller and Smirk, 1932 c].

The percentages of hæmoglobin or of iron in whole blood have a similar significance since the iron is almost entirely present as hæmoglobin. They depend upon the number of circulating red cells and, therefore, are probably influenced by splenic contractions, postural changes in blood volume, alterations in blood-pressure and local changes in the circulation of the part from which the blood sample is taken.

The water content of whole blood as determined by drying is largely controlled by the percentages of hæmoglobin and protein.

Since all the ordinary blood constituents are capable of independent variation, one cannot quite confidently deduce the amount of additional water stored in blood from change in the blood composition. On the other hand, when, after drinking water, a dilution of all the main blood constituents is present, the general blood dilution is most probably due to additional water stored in the circulating blood.

The remaining object was to ascertain if any change in the water content of blood was likely to affect the kidney directly.

Histologically it appears that the blood supplying the kidneys is contained in vessels. There is, so far as I am aware, no evidence that blood is in direct contact with renal tubule cells. The tubule cells are presumably reacting to lymph filtered from blood plasma; alternatively to the constituents of plasma which diffuse through the blood-vessel wall, or possibly to the changes in the composition of glomerular filtrate. If, then, water diuresis is the result of a change in the composition of blood, it must be a change in its diffusible constituents. Again, if the change in composition is one of dilution, rather than the addition or subtraction of some hormone-like substance, then the change is probably a dilution of the salts or electrolytes, or as Haldane expressed it, an increase in the diffusion pressure of water.

Dilution of the blood with a protein-free filtrate of blood would diminish the percentage of hæmoglobin in whole blood and of protein in plasma, and would probably increase slightly the amount of fluid bathing the renal cells—it would not, however, change appreciably the saline composition of this fluid.

On the other hand the withdrawal of salt from the blood—there is abundant evidence that this may occur—would leave the protein-free filtrate of that blood hypotonic; using Haldane's term, the diffusion pressure of water would be increased. Yet the removal of salt from the blood would not necessarily be accompanied by any blood dilution as estimated by the hæmoglobin percentage or the plasma protein, and is not inconsistent with concentration of these latter constituents.

A fall in the salt or electrolyte concentration does not necessarily imply a storage in the blood of any of the water given—it suffices that in its passage through the blood or as a result of the temporary storage of water in tissues some salt should pass out from the circulation.

Experimental procedure.

Doses of drinking water, 1000 to 1500 c.c., were administered by mouth to normal subjects.

Samples of blood were taken before and after the administration of the water. Capillary blood samples were taken from the fingers without compression and venous samples were taken without congestion. The absence of congestion is of considerable importance, since it has been already shown [Smirk, 1928] that changes in the blood composition are induced by congestion alone. Urine samples were taken at intervals, which varied with the nature of the test. The subjects were either

recumbent throughout the tests or else they were engaged in laboratory work and were seated only for short periods.

The following methods of analysis were used:

(1) Changes in the hæmoglobin percentage of whole blood were measured in two ways: (a) By colorimetric comparison of blood samples taken before and after the administration of water. 0.1 c.c. of each blood sample was diluted with 25 c.c. of tap water and converted to carboxy-hæmoglobin by passing coal gas. Using the diluted first sample as standard, the degree of dilution in the second sample was expressed as a percentage of the original blood concentration. (b) The iron content of blood was estimated by the methods described by Smirk [1927 a].

(2) Estimations of the plasma protein were made by Kjeldahl's method without correction for non-protein nitrogen.

(3) Plasma chlorides were determined by rapid destruction of the proteins with ammonium persulphate and nitric acid in the presence of silver nitrate followed by back titration with alcoholic thiocyanate in the presence of acetone as an end-point intensifier [Smirk, 1927 b].

Samples of blood were shaken with air before analysing in order to correct any changes in the distribution of chlorine ions due to differences in the CO_2 contents of the samples.

Heparine was usually employed as anti-coagulant.

RESULTS.

(1) *Control observations.*

(a) *Capillary blood samples.*

Capillary blood samples (0.1 or 0.2 c.c.) were taken at short intervals from pricks in the finger-nail beds made by a bayonet-pointed needle or by Clark's blood gun. The blood should flow freely. The iron p.c. was determined and the results have been recorded graphically on Fig. 1. It will be observed that most results agree closely, but that eight aberrant values are met in thirty-four determinations.

It is a common practice to obtain a more liberal supply of capillary blood by immersion of the hand in hot water. As a control on this procedure samples of blood were taken from the fingers shortly after immersion for 2-5 min. in hot or in cold water and the composition compared with that of samples taken before the hand was either heated or cooled.

Heating or cooling the hand almost always causes some increase or decrease in the hæmoglobin concentration of capillary samples. The

direction of the change is much more frequently an increased hæmoglobin percentage after heating and a decreased percentage after cooling the hand. In one subject the reverse was obtained, and in a few people the results have been irregular.

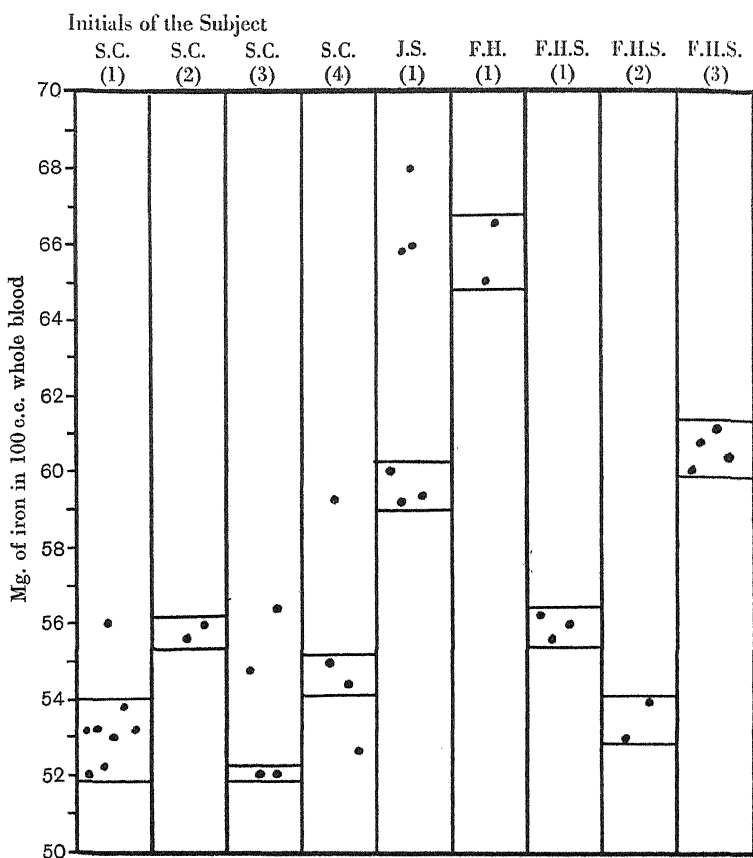


Fig. 1. The normal variability in the composition of capillary blood samples taken from finger pricks.

(b) *The procedure of sampling venous blood.*

Errors resulting from the congesting or heating of a limb from which a venous blood sample is taken. A separate investigation of this subject was made by Smirk [1928].

Congestion increased the concentration of hæmoglobin and caused a transference of chlorine ions from plasma to corpuscles.

Heat produced a venous blood which was bright red and the hæmoglobin content of the samples was sometimes raised.

It was concluded that venous samples taken with momentary congestion were satisfactory if subsequently they were shaken with air to remove CO_2 . If they were not shaken with air the CO_2 content of the blood would vary with the circulation rate through the limb, and so also would the distribution of chlorine ions between plasma and corpuscles.

(c) *An effect of meals on blood composition.*

It was thought advisable to discover whether meals might affect the blood composition in a way which would lead to misinterpretation of results.

In five out of six experiments an increase in the whole blood chloride was observed during the hour following a meal, in which no fluid or salt as such were taken. The whole blood chloride usually returned to normal in 2 or 3 hours.

Dodds and Smith [1923] reported a slight fall in the serum chloride after test meals, but for this they used a pint of liquid. It is possible that my results are due to a dilution of blood with tissue fluid. The increased proportion of plasma would cause an increase in the whole blood chloride.

It is clearly important to wait at least 3 hours after a meal before administering water, as a recent meal might obscure blood dilution from other causes.

(2) *The results of water-drinking experiments.*

The changes in blood concentration after water administration (Figs. 2, 3, 4, 5) have been expressed as a percentage of the blood concentration before giving water. In other words, the initial blood concentration of any substance used as an index of blood dilution is called 100 p.c., and if the concentration of this substance falls to say 96 p.c. of this in the course of an experiment it is recorded as a 4 p.c. dilution. In Figs. 2, 3, 4, 5 the horizontal lines represent the initial blood concentration. The distances of the points below this line represent the degrees of dilution of individual blood samples expressed as a percentage of the average blood concentration before water was given. The vertical line represents the beginning of water administration. The amounts of water given were either 1000 or 1500 c.c. and the time taken to drink this was usually 5 min. The distances of the points to the right of the vertical line indicate the times after giving water at which the blood samples were taken. Figs. 2, 3, 4 and 5 summarize the results obtained

in fourteen experiments on nine subjects. Figs. 6, 7 and 8 give the results of three individual tests.

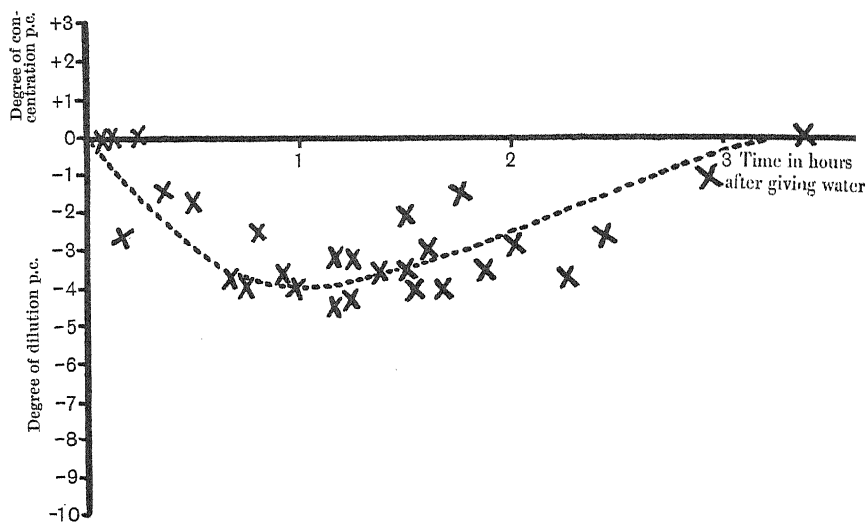


Fig. 2. The effect of water drinking on the whole blood chloride of capillary samples.

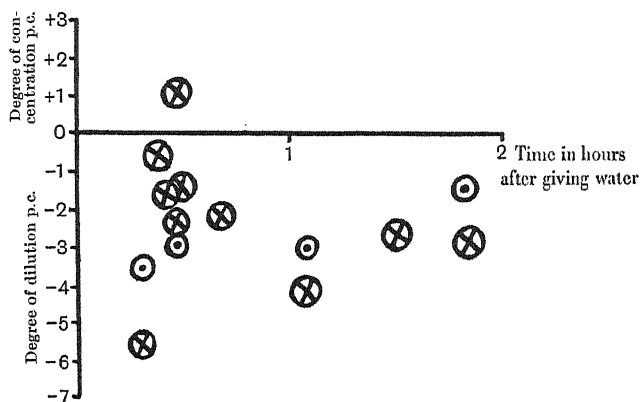


Fig. 3. The effect of water drinking on the p.c. of chloride in the plasma and whole blood of venous samples.

⊗ P.c. dilution of chlorine in plasma. ⊙ P.c. dilution of chlorine in whole blood.

(a) *The effect of the administration of 1500 c.c. of water upon the whole blood chloride of capillary samples.*

Three experiments were made on the same subject. In each experiment (Figs. 6, 7) many samples were taken. The results of the three

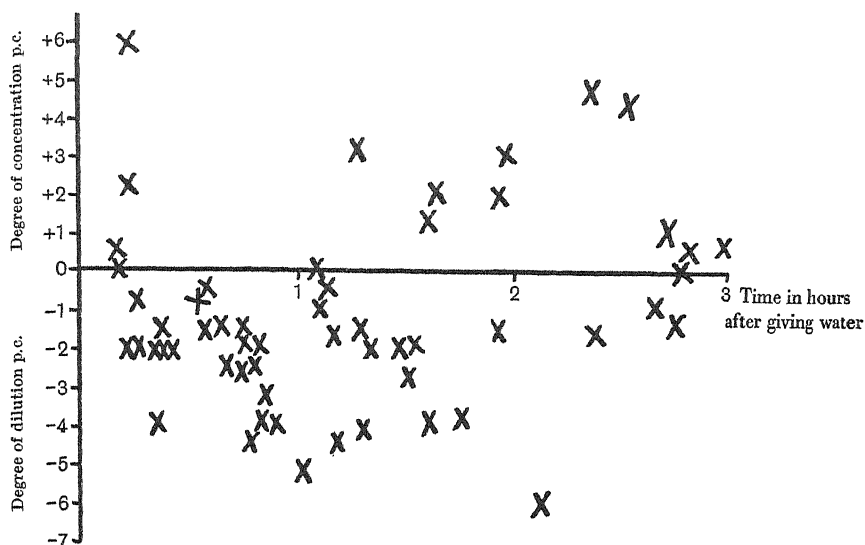


Fig. 4. The effect of water drinking on the p.c. of iron and hæmoglobin in samples of capillary blood.

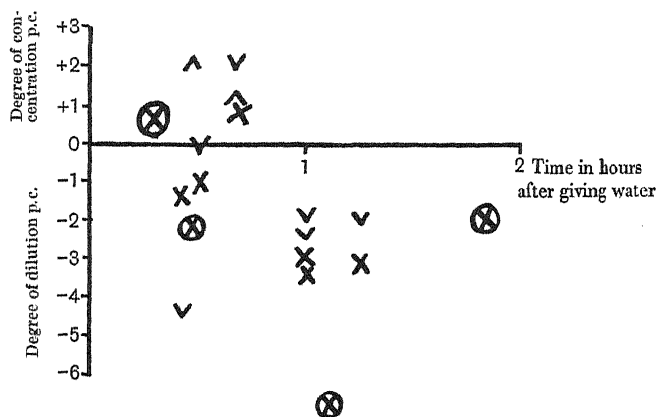


Fig. 5. The effect of water drinking on the p.c. of iron and hæmoglobin in whole venous blood and on the p.c. of protein in the plasma of venous blood.

- ⊗ P.c. dilution of iron in whole venous blood.
- x P.c. dilution of hæmoglobin in whole venous blood.
- v P.c. dilution of plasma protein.
- ^ P.c. dilution of the total solids in whole blood.

experiments are combined in Fig. 2. The chlorine content of whole blood is reduced, and judged by the distribution of the points on Fig. 2, as also by Figs. 6 and 7, the maximum degree of dilution appears at about 40 to 60 min. It will be seen that the maximum diuresis in these experiments was reached about $1\frac{1}{2}$ hours after giving water. Therefore the maximum dilution of whole blood chloride may be attained before the maximum rate of urine formation and, indeed, before the rate of urine formation has been greatly increased.

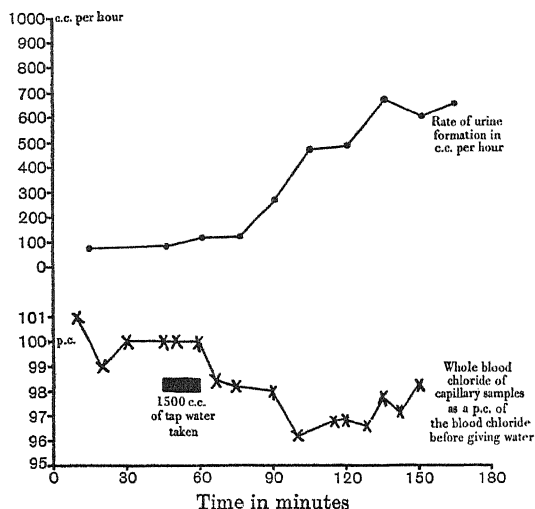


Fig. 6. The effect of water drinking on the whole blood chloride of capillary samples and on the rate of urine formation.

It will be observed also (Figs. 6, 7) that as many as four or five consecutive chlorine determinations are constant within chemical error at the time when the greatest change in the rate of urine formation takes place.

Capillary samples were also taken in a fourth experiment, in which 1000 c.c. of water were given to another subject. Some of the samples in this test showed slight concentration.

The degree of blood chloride dilution is greater than would be expected from an equal partition of additional water among the water-bearing tissues of the body.

(b) *The effect of the administration of 1000 to 1500 c.c. of water upon the plasma chloride and whole blood chloride of venous samples.*

Each point on Fig. 3 is the average of two or more chlorine determinations. It appears that there is a clear dilution of the whole blood and plasma chlorides of venous samples.

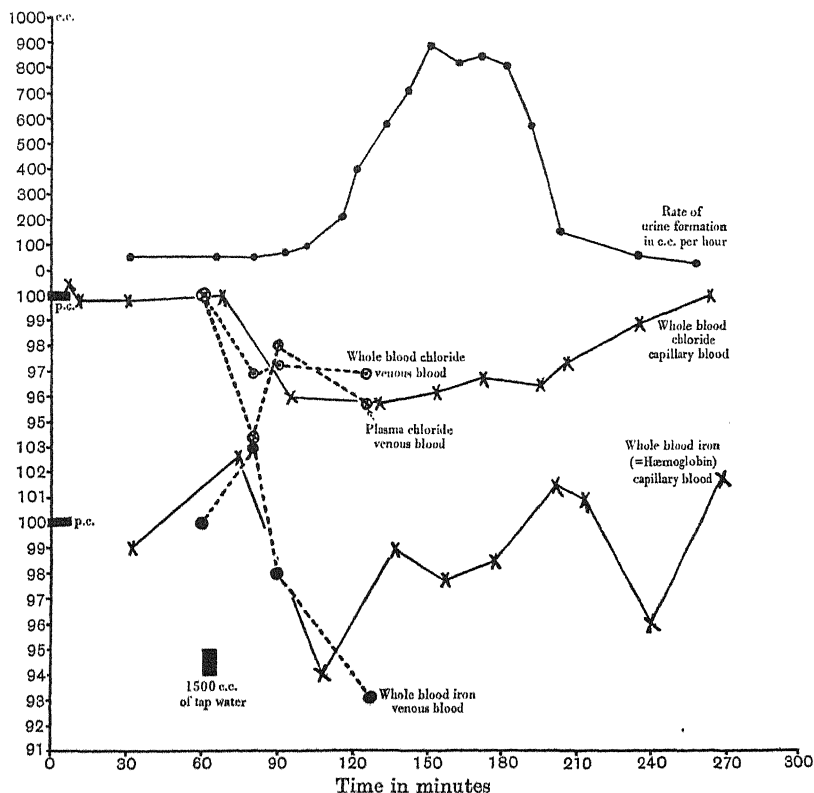


Fig. 7. The effect of water drinking on the whole blood and plasma chloride and on the iron percentage of whole blood, in relation to the rate of urine formation.

The degree of dilution is usually a little greater and sometimes definitely in excess of what would be expected from an equal partition of the additional water among the water-bearing tissues of the body.

Thus, if a 75 kg. man is composed of 60 p.c. water, the uniform distribution of a dose of 1500 c.c. among his water-bearing tissues would produce a blood dilution of about 2.7 p.c., and a dose of 1000 c.c. would produce a 1.8 p.c. dilution (assuming 80 p.c. water in blood).

(c) *The effect of the administration of 1000 to 1500 c.c. of water upon the hæmoglobin percentage of whole blood estimated in capillary samples.*

In Fig. 4 it will be observed that of fifty-five capillary samples removed for hæmoglobin or iron determinations at various times after giving water forty samples show dilution up to 6 c.c. and twelve samples show concentration, but between 20 and 70 min. after giving water twenty-four of the twenty-five samples then taken show dilution. Where

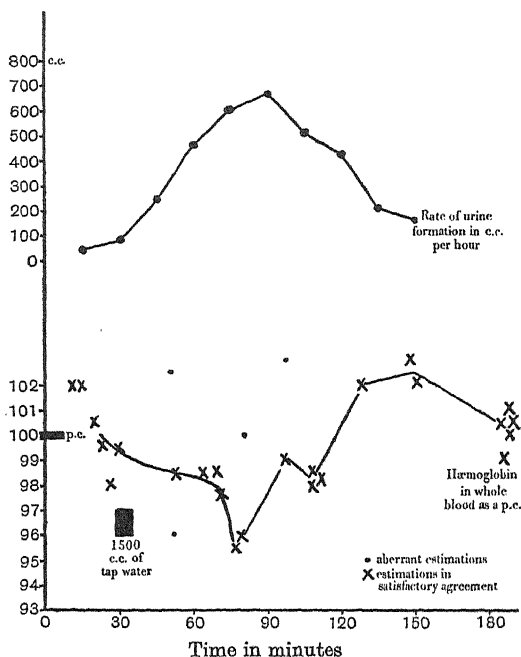


Fig. 8. The effect of water drinking on the hæmoglobin concentration in whole blood and on the rate of urine formation.

several capillary samples have been taken at short intervals of time the range of values obtained has been such as would be expected from the preliminary study of variability in capillary samples (Section 1 (a)); *i.e.* of four or five capillary samples we may expect close agreement in three or four. Applying this to Exp. 14, where a total of twenty-eight capillary samples was taken, four of the capillary samples (see Fig. 8) were clearly aberrant values. It is evident from the agreement of samples taken in triplicate that actual concentration of the hæmoglobin percentage has occurred towards the end of diuresis in Exp. 14 (Fig. 8).

The degree of blood dilution averages about 2 p.c., but may be as great as 6 p.c. It is seen from Fig. 4 and is also evident in the results of individual experiments that the degree of hæmoglobin dilution at any given time bears no proportional relationship to the rate of urine formation at that time.

In Exps. 3, 13 and 14 (Figs. 7, 8), performed on the subject F. H. S., the maximum dilution of hæmoglobin is met about 40 min. after giving water, and this is followed by a reconcentration during the period of rapid urine formation and is succeeded by a slight re-dilution. This is somewhat similar to the changes described by Marx and Mohr in man and in the dog [1927].

(d) *The effect of the administration of 1000 to 1500 p.c. of water upon the hæmoglobin percentage of whole blood, the dried weight of whole blood and the protein nitrogen of plasma estimated in venous samples.*

Fig. 5 shows that where venous samples are used an increased concentration in hæmoglobin and in the dried weight of whole blood (which depends mainly on hæmoglobin) is met in the first half hour after water administration. One hour after giving water a blood dilution of 2 to 3.5 p.c. appears to be the normal rule. Only two dilutions out of a total of eighteen observations are more than 4 p.c.

The initial increase in concentration of the non-diffusible blood constituents is probably not of methodical origin, the subsequent slight general blood dilution is, I think, unquestionably present in most experiments after water drinking.

DISCUSSION.

In the rat, guinea-pig and rabbit the relationship between water absorption, storage and diuresis has been considered by Heller and Smirk [1932 *a*, 1932 *b*], and between blood composition and diuresis in the rabbit [Smirk, 1932]. In the rabbit diuresis seemed unrelated to the degree of general blood dilution as estimated by the hæmoglobin percentage, the dried weight of whole blood and the plasma protein percentage. In the rat and also, though less certainly demonstrated, in the guinea-pig, water absorption is well in advance of diuresis, so that the maximum load of absorbed but as yet unexcreted water precedes the maximum diuresis by as much as 20–30 min. Also in the preceding paper it has been shown that there is a similar relationship between the water load and diuresis in man. It appears that, at any given moment, water diuresis is not proportional either to the degree of general blood dilution or to the excess of water then present in body tissues.

The partition of water between blood and tissues.

It is beyond question [Priestley, 1921 and others] that the changes which occur in blood composition after water drinking are small compared with the enormously increased output of urine. My results provide examples of the slight degrees of dilution obtained by Haldane and Priestley [1916] and, though less frequently, of the relatively great dilutions recorded by Marx and Mohr. It has been shown in the control observations that the reliability of any single capillary sample must be questioned, but where a consistent change has been observed in many capillary samples there is little chance of methodical error explaining the results. In venous samples methodical error is minimized when two or more blood constituents are used as indices of dilution, and simple chemical errors are avoided by duplicate or triplicate analysis.

I consider that in most of my experiments the variable blood dilution as determined by the analysis of capillary and venous samples represents an actual variability in the degree of dilution in circulating blood. The variations depend upon the subject of the experiment, the time after drinking water at which samples of blood are taken and upon the rates of absorption and diuresis. A similar conclusion was reached in rabbits.

Using capillary samples and hæmoglobin or iron as an index of blood dilution, a fall in hæmoglobin percentage is not invariable and concentration may be observed, as also in venous samples using the hæmoglobin percentage and the plasma protein. In both capillary and venous samples an increase in the hæmoglobin percentage is not infrequently met a few minutes after swallowing water. This rise in hæmoglobin was reported by Marx and Mohr. It has also been observed in the experiments where pituitrin was given subcutaneously in addition to the water. The plasma chloride was regularly diluted to between 1 and 6 p.c. The whole blood chloride was diluted in twenty-eight out of thirty-two samples, taken after water administration and four out of the four samples which failed to show dilution occurred in the same experiment. A slight increase of the chlorine content of whole blood is not inconsistent with chlorine dilution in plasma. (See Introduction.)

The degree of dilution measured both by diffusible and non-diffusible constituents is frequently greater than would be expected if all water given was absorbed and distributed uniformly throughout the water-bearing tissues of the body. This is still more convincingly illustrated in some of the experiments in the subsequent paper where diuresis has been prevented by pituitary hormone. One may quote here the results of

Margaria [1930] who observed a fall in the vapour pressure of blood after water drinking. The change in vapour pressure (expressed in terms of a sodium chloride p.c.) indicates a degree of dilution which is greater than would be expected if the water had been equally distributed among the water-bearing tissues of the body. Is this increased degree of dilution the result of delay in attaining equilibrium, or is the treatment of a new supply of absorbed water different from that of water already present and well established in body tissues? In other words, is only a part of the body water in a "labile" or "free" state so that the addition of a fresh supply of water produces a greater effect than would be expected from its proportion to the total water.

When the excretion of water is prevented by pituitrin hormone the excess of water in the blood is even greater, and a period of usually 40 or 50 min. passes before the blood has reconcentrated to something about what would be expected from an equal partition of water. If we assume that this degree of blood dilution is merely the expression of a time lag in the disposal of water, then one must comment upon the slowness with which equilibrium is established under certain circumstances.

In this respect Hill [1930 b] has shown that if we define "free" water as the weight of water in 1 g. of fluid or tissue which can dissolve substances added to it with a normal depression of vapour pressure, then practically all of the water in blood (98 or 100 p.c.) and 77 p.c. of the total 80 or 81 p.c. of water in frog's muscle is free. The preliminary experiments of Margaria have shown that at least part of a fresh supply of water absorbed from the alimentary canal also remains "free" to depress the vapour pressure. It would appear that any distinction there might be between a new supply of absorbed water and the water already present in tissues is not likely to be the "free" or "bound" state as defined by the power to dissolve substances with the normal depression of vapour pressure.

The relationship between water absorption and blood dilution.

As the distribution of extra water between blood and tissue varies, quantitative deductions as to the amount of water absorbed by any individual subject could not be made from changes in blood composition after water drinking. Yet it is evident in Exps. 1, 2, 3 and 4 where several blood samples have been taken that the maximum degree of whole blood and plasma chloride dilution is met as frequently in the second half hour after giving water as at any subsequent period. It has been shown in the previous paper that a litre of warm water taken by mouth probably will be

absorbed in 30–50 min. There is also some evidence that the water is more rapidly absorbed when the subject is fasting and in a sitting posture.

The maximum or almost the maximum chlorine dilution is usually met before or about the onset of diuresis. This has also been recorded by Rioch [1927, 1930] on dogs and on man. The electrical conductivity was used as an index of dilution and the changes observed may be fairly taken to indicate a diminished concentration of the chief electrolyte sodium chloride.

But the dilution of the diffusible chlorides may be produced as easily by chlorine leaving as by water entering the blood. The absorption of water and its storage in tissues may cause salt to be withdrawn from the blood in order to maintain this stored water in a more nearly isotonic condition. The storage of water in tissues after water administration has been demonstrated by Baer [1927], Tashiro [1926], Heller and Smirk [1932*a*]. If this alone caused chloride dilution the attainment of maximum dilution before diuresis is fully established would agree with the conclusion already drawn, that a large part of the administered water is absorbed before the urinary output is greatly increased. There remains, however, an alternative explanation for part of the chloride dilution; chlorides may enter the water in the alimentary canal prior to its absorption. In some experiments on rats in which 5 p.c. body weight of warm water was administered by stomach tube the chloride percentages in the residual water at a time when absorption was nearly complete were as follows: in the stomach 0.1 p.c. NaCl and in the intestine 0.55 p.c. NaCl. The total amounts of chlorine involved were calculated approximately. Translated into human terms they would be sufficient to alter measurably the chlorine content of blood. We are not aware of the relative surfaces of alimentary mucous membrane per kg. body weight in the rat and man, and it is probable that passage of chlorine to the water in the alimentary lumen plays a smaller part in man. Controls showed that about half the chloride found might be accounted for by chloride already present in the alimentary tract. Nevertheless, this process may explain part of the chloride dilution. The hæmoglobin and plasma protein percentages are uninfluenced however by migration of chlorine into the intestinal contents. In Fig. 7 it will be observed that degrees of dilution recorded are as great in the second half-hour period after water administration as at any subsequent period. This observation is consistent with the conclusion that a considerable absorption of water has taken place before diuresis is established (see the preceding paper).

The relationship between blood composition and urinary output.

It will be clear from Figs. 3 to 8, and Exps. 1 to 12 also show that the maximum degree of blood dilution is usually attained between 30 and 60 min. after giving water as would be expected from the duration of water absorption (see the previous paper). At this time the rate of urine formation has increased but slightly, the maximum rate being attained 20 or 30 min. later. If then the maximum dilution both of diffusible and non-diffusible blood constituents occurs usually some 20 min. before the maximum diuresis, then at any given moment the rate of urine formation will not be proportional to the degree of blood dilution then present. This is the equivalent of the results of Rioch who observed that the plasma chloride is roughly in inverse proportion to the diuresis if we make allowances for a lag of some 20-30 min. between the changes in blood and urine. He concludes from this that the most probable cause of water diuresis is the electrolyte dilution.

While supporting the observations of Rioch for many reasons I do regard this parallelism with a lag as evidence that the electrolyte dilution and water diuresis are causally related. For, once a dilution of blood has been established as a result of water absorption, the degree of dilution may be expected to diminish as the excess of water is excreted. In other words, the mere fact that absorption is in advance of diuresis explains the dilution of various blood constituents and the presence of a lag between blood dilution and diuresis.

Nevertheless, for reasons outlined in the Introduction, it seems that any change in blood composition which is to influence the kidney cells is probably a change in the diffusible constituents of blood. It has been shown, however [Heller and Smirk, 1932 b], that if rats are depleted of 5 p.c. body weight of water or rabbits of 4 p.c. body weight of water and then this water is given back to them by stomach tube, a diuresis ensues which is not much less than that of control animals receiving 5 or 4 p.c. body weight of water without previous depletion. What is it that determines the typical water diuresis in these animals? There is no excess of water in the body as a whole, and so far as we can tell there has been no excretion of salt. Salt excretion would account for electrolyte dilution if the salt and water lost were replaced by water alone. It is said that rabbits do not sweat, and it certainly appeared in our experiments that the water was excreted through the respiratory system. It may be said that, although in these experiments there is no excess of water in the body as a whole, an excess is present in the blood and this determines

diuresis. Clearly the initial electrolyte dilution could start a diuresis; the difficulty is to explain why it should continue when this temporary excess of water in the blood has been excreted by the kidneys. At this stage there is no excess of water either in the blood or in the tissues, and so far as one can say there is likewise no electrolyte dilution either in the plasma or in the tissues.

I do not consider that this experiment invalidates the theory that electrolyte dilution or an increase in the diffusion pressure of water is the cause of diuresis. It might well be that it is the change in electrolyte percentage rather than the absolute concentration which determines diuresis. As an analogy may be quoted the action of muscarine upon the heart in which it is the passage of the drug into and not the presence within the cell substance which determines its activity.

In man the loss of water by sweating is accompanied by a marked reduction in the urinary output. But in sweating both water and salt are lost. The conditions, therefore, are not the same as in the water-depletion experiments on rats and rabbits. It is highly probable, however, that the processes leading to water diuresis are essentially similar in mammals. This difficulty in accepting the absolute level of the diffusion pressure of water as the factor controlling water diuresis applies equally whether we consider that it operates upon the kidney directly or by some indirect mechanism such as the removal of the inhibitory influence of the pituitary hormone [Verney, 1929]. Verney's hypothesis does not assume, of course, that changes in the absolute level of the water-diffusion pressure control the activity of the pituitary gland.

But unless these experiments are explained by some factor which we have overlooked, it would appear that any satisfactory theory concerning the mechanism of water diuresis must ultimately show how in these animals, with no excess of water, the body is able to distinguish between water that has been well established in the body and a fresh supply of water. Why is it that in rats and rabbits a fresh supply of water is excreted although it is manifestly needed to replace water already lost, and it is eliminated at a time when there is no excess of water in the body?

Perhaps when water is first absorbed it is not in the same "state" as when it has been in the body for some time. For example, there may be a relatively slow penetration to within the tissue cells, or a time interval before the new water is taken up by colloids, so that this new water may be more available or perhaps is more in the tissue spaces and less in the cells. For a period of time it can perhaps exert some function which well-established water can perform less readily. Thus its presence might

influence the tissue of the pituitary gland and check the secretion of its anti-diuretic principle. Alternately a fresh supply of water may pick up some hormone-like body which is directly or indirectly the cause of the renal response and would explain why, in this particular case, the response is independent of the immediate needs of the organism.

These speculative suggestions are yet, and may remain, without foundation. They perhaps serve the purpose of indicating more clearly the nature and difficulties of the problem: why is much dilute urine passed after drinking water?

SUMMARY.

1. In man the drinking of 1 to 1.5 litres of water is usually associated with dilution of the blood as estimated by the plasma chloride, whole blood chloride, whole blood iron, hæmoglobin and plasma protein.

2. The maximum degrees of dilution are found as early as 30–60 min. after giving water. This agrees with the previous conclusions as to the time expended in water absorption.

3. The fall in concentration of various blood constituents is often greater than would be expected from an equal partition of water among the water-bearing tissues of the body.

4. When blood dilution is determined by estimating the chloride or hæmoglobin percentage in whole blood or by the protein in plasma, there appears to be no relationship between the rate of urine formation and the degree of blood dilution present at the time when the urine was formed.

5. Together with previous work on animals these observations throw light upon, and are discussed in relation to, the cause of water diuresis.

I wish to offer my sincere thanks to Prof. Craven Moore for his stimulating encouragement and interest in the early stages of this work, and to Prof. Verney for a valuable criticism.

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THE INFLUENCE OF POSTERIOR PITUITARY
HORMONE ON THE ABSORPTION AND
DISTRIBUTION OF WATER IN MAN.

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INTRODUCTION.

It is well known that in man as in lower animals the absorption of a small dose of pituitary hormone injected subcutaneously will prevent the diuresis which usually follows a large dose of water, but the exact cause of this prevention of diuresis has not been demonstrated conclusively. Rees [1920], for example, working on dogs, found a delay in the water absorption when pituitary hormone was given previously.

The object of the work here recorded was the study in man of the alimentary absorption rate of this water, together with its partition between blood and tissues.

EXPERIMENTAL PROCEDURE.

The time expended in the alimentary absorption of water at 37° C. has been determined by the methods described in the first of the preceding papers. The water partition between blood and tissues has been deduced from the changes observed in the composition of the blood, assuming that the absorption rates so determined are correct. Numerous samples of blood, usually from finger pricks, were taken before and after drinking water. The methods of blood sampling and of analysis used and the experimental precautions needed are described in the second of the preceding papers. The dose of pituitary hormone was usually 0.05 to 0.1 c.c. pitressin subcutaneously. This dose always delayed the onset of diuresis for several hours.

RESULTS.

(a) *The influence of pituitary hormone upon the time taken in the absorption from the human alimentary canal of 1 litre of warm water.*

In most of the experiments the rate of water absorption has been determined by the method of weighing the legs, but in one experiment the "abdomen weighing" method was used. The results are given on Figs. 1-8, and these results may be compared with similar experiments

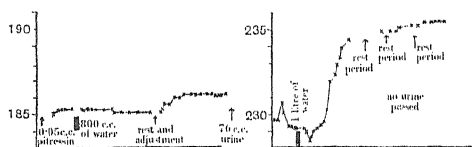


Fig. 1. Subject A.N.

Fig. 2. Subject F.H.S.

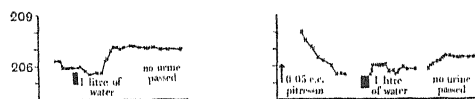


Fig. 3. Subject McM.

Fig. 4. Subject McL.

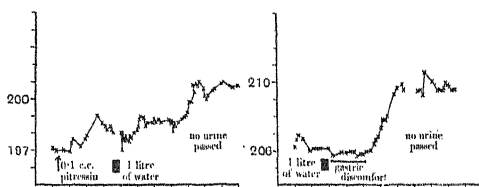


Fig. 5. Subject F.H.S.

Fig. 6. Subject W.N.

Figs. 1-6. Changes in the weight of the legs after drinking water when diuresis is prevented by pituitary hormone. Abscissa: Time of experiment (10 minute intervals). Ordinate: Weight in g. needed to counterpoise the legs.

in the first of the preceding papers, where only water was given. Often the same subjects were used.

If we assume that absorption is practically complete when the weight of the legs has ceased to increase it is evident that, so judged, the rate of water absorption is not greatly influenced by pituitary hormone. The estimated rates of absorption with and without pituitrin are compared in Table I. They are similar to the absorption rates as deduced from the changes in blood composition described in the second of the preceding papers.

The alterations in leg weight after pituitrin and water differ in one

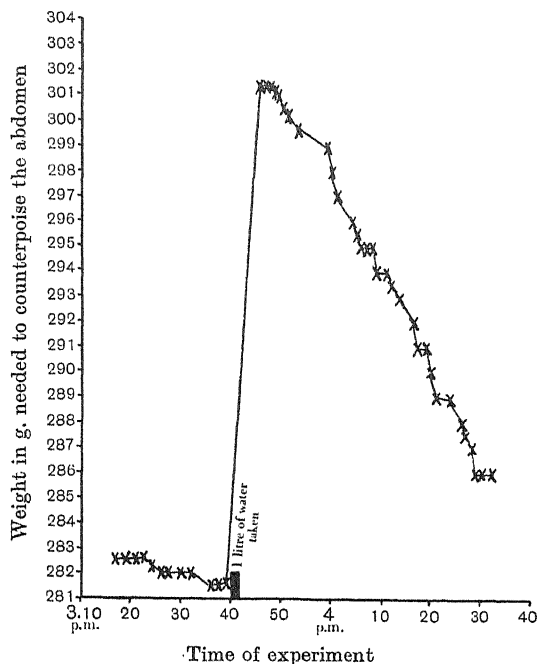


Fig. 7. Changes in the weight of the abdomen during and after drinking water when diuresis is prevented by pituitary hormone. Subject F.H.S. (Pitressin).

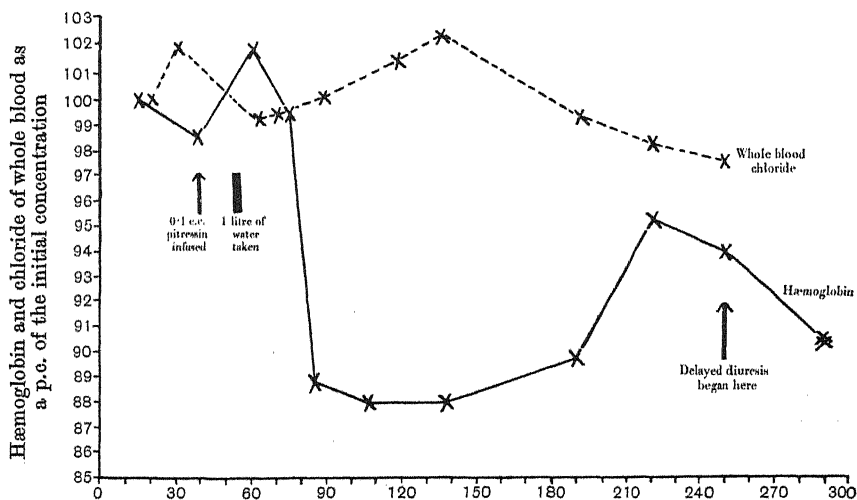


Fig. 8. Changes in the haemoglobin and chloride concentrations of whole blood after water drinking when diuresis is prevented by pituitary hormone. Subject McM.

way from the changes which follow simple water drinking. Where no pitressin is given the onset of urine formation is accompanied by a fall in the leg weight, owing to the withdrawal of water from the limb. If pitressin is given the rate of urine formation is not increased by giving water, and the increase in leg weight resulting from the absorption of water is maintained. This provides additional evidence that the changes in leg weight are mainly the result of water absorption. An experiment where the rate of absorption was determined by the method of weighing the abdomen confirms these conclusions (Fig. 7).

TABLE I. The influence of pituitary hormone on the absorption rate of water.

Subject	Controls without pitressin. Time taken to absorb 1 litre of warm water (min.) as determined by		Experiments with pitressin. Time taken to absorb 1 litre of warm water (min.) as determined by		
	Leg weighing method	Abdomen weighing method	Leg weighing method	Abdomen weighing method	Blood dilution method
A. N.	28	40	55	—	—
	55	35	—	—	—
	45	—	—	—	—
	25	—	—	—	—
F. H. S.	30	55	23	45 (approx.)	40
	50	27	50	—	60-90
	35	22	40	—	50
McM.	45	—	25	—	50
	—	—	—	—	40
	—	—	—	—	25
McL.	—	—	45	—	—
R.M.	50	—	—	—	—
P.F.	—	—	—	—	50
W.N.	—	—	45	—	—

(b) *The effect of drinking 1 litre of water upon blood concentration when diuresis is prevented by pituitary hormone.*

Relatively large doses of pituitrin administered to animals produce a fall in the hæmoglobin percentage, but as Poulsson [1930] also observed smaller doses administered to human subjects cause less constant changes in blood composition. It is generally agreed that the much larger degrees of blood dilution obtained when both water and pituitrin are given are due to the presence of additional water in the blood and not to changes induced by giving pituitrin.

So long as the rate of water absorption from the gut exceeds the rate of its storage in tissues, the water content of the blood will continue to increase and dilution of the hæmoglobin and protein will be observed.

It remains to be considered whether we can make any deduction as to the water absorption or storage rates by following the progress of blood dilution.

In most of the experiments the capillary blood samples taken before water but after pituitrin was given show a range of variation greater than that observed in the control observations of Section 1 *a* in the second of the preceding papers, which concerns the range of variability in capillary samples. This increased variability is to be associated perhaps with the blanching of the skin. In nine out of ten experiments there is no doubt that, after water is taken, a marked dilution of blood occurs, the maximum dilution being attained about 30–60 min. after drinking the water. This degree of dilution tends to be maintained for a variable period and is followed by a return of the hæmoglobin percentage towards, and sometimes to, its original concentration. The maximum degrees of dilution recorded are clearly greater than would be obtained by an equal partition of the extra water given among the water-bearing tissues of the body. The re-concentration has also been observed by Poulsson [1930] and is presumably due to the passage of water into the tissues, since when re-concentration occurs there has been no increase in the urine formation. Presumably as the blood becomes diluted, part of the water passes on to the tissues; otherwise the degree of blood dilution would be greater. Up to a period, however, of 25–50 min. after giving water the absorption rate exceeds the storage rate of water in tissues. It would appear most reasonable to assume that at first the rate of water absorption is greater than the rate at which water leaves the blood for the tissues. But, as absorption comes to an end, the passage of water into the tissues causes a partial re-concentration of the blood, until finally water is divided equally among the water-bearing tissues. The observations in the first of the preceding papers on the rate of water absorption lend their support to this explanation. It is not improbable that the extra water leaves the blood more slowly when pituitrin has been given, as is suggested by the experiments of Sachs [1924] on cedema formation.

It will be seen that there is frequently a delay of 10 or more minutes after water drinking before the onset of hæmoglobin dilution, and a temporary blood concentration may occur just as when no pituitrin is given. Again in Section (*a*) of this paper and in the first of the preceding papers it has been shown that frequently the leg weight does not begin to increase until 10 or even 20 min. after giving water. These results indicate a short delay before the onset of water absorption in man.

DISCUSSION.

Although the results of Section (a) do not allow us to state that the rate of alimentary absorption of water is quite uninfluenced by pituitary hormone they, at least, suggest that it has no great or constant influence, and that in man it is unlikely that any appreciable part of the anti-diuretic action of pituitary hormone is the result of a reduced absorption of water. The evidence provided in Section (a) is strongly supported by the changes in blood composition recorded in Section (b) in which it is shown that, after giving water to human subjects who have previously received an injection of pitressin, the blood is progressively diluted for a period ranging from 35 to 50 min. after giving water. The time taken to obtain a maximum degree of blood dilution is approximately equal to the time expended upon water absorption as determined by the methods of weighing the legs and abdomen: and this again covers the same range as the normal times for the absorption of 1 litre of water already described in the first of the preceding papers. The dose of pitressin used for human subjects was much less per kg. of body weight than the dose employed for rats by Heller and Smirk [1932 c], but in so far as pitressin did not influence the water absorption rates the results are comparable, and together may be contrasted with the observations made by Rees on dogs.

We must now consider the manner in which this absorbed, but as yet unexcreted, water is distributed between the blood and the tissues. For reasons that have been explained in Section (a) and in the first of the preceding papers, the degree of increase in leg weight cannot be regarded in any single case as an accurate indication of the percentage increase in the amount of stored water. Nevertheless, an average of seven results suggested an increase of 1.15 p.c. in leg weight, which is a sufficient approximation to the degree of increase (1.3 p.c.) which would be expected from the amount of water given. Likewise, in the subjects receiving pitressin, the individual percentage increases vary considerably, but the average is 1.28 p.c.

The closeness of this percentage to the theoretical increase is quite accidental, for the method does not admit of anything like this accuracy. The increase is, however, of the order expected. Again, in the single abdomen-weighing experiment, the increase in the weight needed to counterpoise the water consumed was only a little greater than the decrease observed during water absorption.

It is clear, however, that since the water given is absorbed and not excreted, practically all the water must be distributed between the blood

and the tissues, so that if we estimate the increased amount of water in the blood it will enable us to determine the approximate amount that must be stored in the tissues. As determined by the hæmoglobin percentage the amount of extra water in the blood varies considerably from case to case and in the same case at different times. If we take the maximum dilution recorded (12 p.c.) and assume a blood volume of about 5 litres the amount of additional water held in the blood must be about 680 c.c.; the remainder must be in the tissues, lymphatic vessels or serous cavities of the body. In general, however, the degree of blood dilution is much less than this, and usually the amount of extra water held in the blood is about 300 to 400 c.c.

Such calculations show that the water given cannot be accounted for in any instance by the amount of extra water contained in the blood; but in all cases the percentage increase in the water content of blood at the height of dilution is greater than that in the remaining tissues of the body taken together. We can, therefore, state that the anti-diuretic action of pituitary hormone is exerted at a time when the water given has been absorbed and is present in excess both in the blood and tissues. This finding in man resembles that obtained by Heller and Smirk on rabbits, in which an increase in the water content of blood and of muscle was demonstrated directly and in which an increase in the fluid content of serous cavities was found. It appears for this reason that the absence of diuresis cannot be explained either by a shortage of water in the tissues or in the blood, or [Heller and Smirk, 1932 c] by any increase in the avidity of the plasma for water. From the work of Starling and Verney [1925] and Verney [1926] it is beyond question that the pituitary hormone prevents the polyuria of perfused isolated kidneys by a direct renal action. It would seem most likely, therefore, that the prevention of water diuresis by pituitary hormone is also mainly renal and not extra renal in origin. These observations on man, together with the previous experiments on rats and rabbits, are consistent with this explanation.

CONCLUSIONS.

1. When diuresis is prevented by pituitary hormone (0.05 to 0.1 c.c. pitressin) the absorption of 1 litre of water takes place normally. The extra water causes an increase in the leg weight, which weight is maintained at about its maximum level for a longer period than in the experiments where diuresis is allowed to take place normally.

2. The degree of general blood dilution is at first usually much greater than would be expected from equal partition of water among

the water-bearing tissues of the body: and whereas the degree of dilution in blood has averaged 7 p.c., the degree of dilution in the tissues of the leg has averaged about 1.3 p.c. (assuming 40 p.c. solid substance).

3. The spontaneous re-concentration of blood which has been diluted as a result of giving pituitary hormone and water is due to the continued transference of water from blood to tissue, lymph or serous cavities at a time when absorption is ended.

4. In man the absence of diuresis after pituitary hormone cannot be attributed either to delayed absorption of water or to the excessive removal of water from the blood by the tissues. Nor can it be attributed to general delay in the passage of water from blood to tissue, since there is clear evidence of an excess of water in the tissues.

I wish to thank Dr McMichael for his generous help in several of the experiments.

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NOTE ON THE SUBCUTANEOUS ABSORPTION
OF OILS BY RATS AND MICE, WITH SPECIAL
REFERENCE TO THE ASSAY OF ŒSTRIN.

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OIL has often been used as a medium for the subcutaneous administration of substances insoluble in water, or most effective when absorbed slowly. Crude preparations of the gonad hormones are themselves oily, while purer or crystalline preparations are frequently dissolved in oil for injection. Olive, sesame and castor oils are most commonly used for this purpose. During some recent work on œstrin in castor oil and testis hormone in sesame oil it became apparent that absorption was very incomplete. The injected animals were therefore carefully skinned and the subcutaneous tissue examined. Pockets of oil were found to persist for many days (sesame oil) or even weeks (castor oil) after the injection of quite small amounts. The experiments described below were then carried out.

METHODS.

Varying amounts of olive, sesame and castor oils were injected into rats and mice. After the first few experiments, the oils were coloured with Scharlach R to assist detection at autopsy. The injections were made with a fine needle under the skin of the middle of the back. The animals were killed at intervals after injection, and the skin removed with the subcutaneous tissues attached, fixed and preserved in formalin. Careful dissection was required to avoid bursting the pocket of oil. The amount of oil remaining at autopsy was estimated approximately by comparison with known quantities injected into an animal which was killed immediately afterwards.

The experimental results are shown in Table I.

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TABLE I. Subcutaneous absorption of oil.

Animal	Oil given (c.c.) Olive oil	Time of killing after injection. Days	Approximate proportion of oil remaining at autopsy
Mouse L 1	0.1	2	$\frac{1}{2}$
" L 2	0.1	5	Trace
" L 3	0.1	7	0
" L 4	0.2	7	$\frac{1}{2}$
" L 5	0.5	14	$\frac{1}{4}$
Rat L 1	0.1	2	$\frac{1}{4}$
" L 2	0.2	7	$\frac{1}{4}$
Sesame oil			
Mouse S 1	0.1	4	$\frac{1}{4}$
" S 2	0.2	4	$\frac{1}{2}$
" S 3	0.2	7	$\frac{1}{2}$
" S 4	0.5	14	$\frac{1}{3}$
" S 5	0.5	25	$\frac{1}{4}$
" S 6	0.5	56	$\frac{1}{4}$
" S 7	0.5	56	$\frac{1}{4}$
Rat S 1	0.1	2	$\frac{3}{4}$
" S 2	0.1	4	$\frac{1}{4}$
" S 3	0.2	3	$\frac{1}{2}$
" S 4	0.2	6	$\frac{1}{4}$
" S 5	0.2	7	$\frac{1}{4}$
" S 6	1.0	2	$\frac{3}{4}$ +
" S 7	1.0	14	$\frac{1}{2}$
Castor oil			
Mouse C 1	0.05	7	$\frac{3}{4}$
" C 2	0.1	4	$\frac{3}{4}$ +
" C 3	0.1	27	$\frac{3}{4}$ +
" C 4	0.1	27	$\frac{3}{4}$
" C 5	0.1	28	$\frac{1}{2}$ +
" C 6	0.1	28	$\frac{2}{3}$
" C 7	0.2	4	$\frac{3}{4}$ +
" C 8	0.5	19	$\frac{1}{4}$
Rat C 1	0.1	6	$\frac{1}{4}$
" C 2	0.1	7	$\frac{1}{2}$
" C 3	0.2	14	$\frac{1}{4}$
" C 4	0.5	2	$\frac{3}{4}$ +
" C 5	0.5	16	$\frac{3}{4}$
" C 6	1.0	14	$\frac{3}{4}$ +
" C 7	1.0	23	$\frac{3}{4}$

In view of the nature of the experiment, the results are reasonably consistent, except for the quite exceptional disappearance of castor oil in Mouse C 8.

The following conclusions may be drawn:

(a) The three oils dealt with are absorbed very slowly, castor oil, in particular, being remarkably persistent. Olive oil is absorbed rather better than sesame oil by both rats and mice.

(b) Considered in terms of gross amounts all three oils are absorbed more readily by rats than by mice; in relation to body weight, however,

less oil is absorbed by rats. This is no doubt due to the fact that the amount of blood and lymph circulating round a globule of oil of a given size will not necessarily be greater in a larger animal.

(c) With sesame oil or olive oil injections there is a possibility of 0.1 c.c. in mice and 0.2 c.c. in rats being absorbed within a week, but larger amounts of sesame oil or even small amounts of castor oil appear to leave residues almost indefinitely.

(d) A greater gross amount is absorbed when a larger amount is injected, but it is not possible to say whether the quantity absorbed in a given time is proportional to the quantity injected. Other things being equal, the rate of absorption might be expected to depend on the surface area and, therefore, on both the volume and the shape of the globule.

RECOVERY OF OESTRIN FROM UNABSORBED OIL.

The above results make it clear that these oils must be highly unsuitable media for use in the quantitative assay of hormones. This is especially true where the reaction produced is normally rapid and clear-cut, such as vaginal cornification, the usual criterion of oestrus-producing activity. In the assay of oestrin it is essential that the hormone is present in the circulation long enough to induce detectable, but not unduly prolonged, cornification. An oily medium is often used to secure this result without multiple injections, but our experiments show that, if the hormone is absorbed with and proportionately at the same rate as the oil, absorption would be extended over an absurdly long period. It follows that the use of oil as a solvent in the quantitative assay of oestrin would only be justified if the hormone was absorbed rapidly from the more resistant oil. This is inherently unlikely with a substance as insoluble in aqueous media as oestrin. We were, therefore, led to determine whether activity remained in unabsorbed oil after cornification had occurred and passed off¹. Our first experiment was to massage the skins of sixty injected mice in the hope of causing further absorption and a second oestrus in the same animals. No positive results were obtained, and a number of autopsies showed that the treatment had been comparatively ineffective in disturbing the unabsorbed oil. Subsequently, we carried out the following experiment: 0.1 mg. trihydroxy-oestrin in 0.2 c.c. castor oil was injected into each of seven ovariectomized mice. When oestrus had occurred and passed off, the animals were killed and as much

¹ Prof. E. A. Doisy had previously pointed out to us that in his experience a second oestrus might be produced if the unabsorbed oil was disturbed by a second (blank) injection.

as possible of the unabsorbed oil collected and injected into fresh ovariectomized animals. The results are shown in Table II.

TABLE II. Activity of unabsorbed castor oil solution of œstrin.

Exp.	Duration of œstrus in first mouse. Days	Time of killing of first mouse after injection. Days	Result of injection of unabsorbed oil into second mouse
1	3	5	+
2	1	3	-
3	4	8	-
4	7	11	+(weak)
5	7	11	+(weak)
6	11	15	+
7	11	15	+

It will be seen that activity was found in the unabsorbed oil in five of the seven experiments. One of the two experiments giving negative results was vitiated by the fact that only a trace of oil was recovered from the first mouse for injection into the second animal. These experiments show conclusively that, not only is the oil itself not absorbed, but that detectable amounts of œstrin remain behind in it even after a fortnight. It is not possible to give much indication of the amount of hormone remaining in the unabsorbed oil in these experiments, but from the facts (*a*) that recovery of the unabsorbed oil must have been very incomplete, and (*b*) that 10 γ in 0.1 c.c. castor oil had previously been found to give cornification in only thirty out of forty mice, it must be supposed that an appreciable proportion of the original activity may have been present in the unabsorbed oil. A further point arises in this connection. The vaginal epithelium in the ovariectomized mouse will remain cornified indefinitely if an adequate supply of œstrin is available, and the duration of cornification in the first mice used in this experiment is therefore of interest. Since cornification ended while an amount of œstrin capable of causing œstrus in other mice was still present in the unabsorbed oil, it is necessary to suppose that the rate of absorption of the hormone falls below the effective level while adequate amounts are still present.

DISCUSSION.

The experiments described above definitely disprove the supposition that œstrin is absorbed rapidly and quantitatively from a slowly absorbed medium such as castor oil, and this conclusion is probably true for similar hormones in similar media. For other hormones, such as that of the testis, requiring longer administration to produce their effect, the

slowness of the absorption in oily medium would be less fatal to assay, especially if sesame or olive oil were used. On the other hand, giving as little as 0.2 c.c. daily to rats, several days would be required after the last injection for absorption to be completed.

In this connection, Korenchevsky, Dennison and Schalit [1932] state that "...when the testicular hormone is injected dissolved in oil uncertain amounts of unabsorbed oil were always found in the subcutaneous tissue at the places of the more recent injections. This probably indicates that some of the hormone injected during the assay was not absorbed. The amount of this unabsorbed hormone in oil may vary and become a source of error. In our experiments an attempt to decrease this error was made by increasing the period of injections to 10 days, thus allowing time for the complete absorption of the extract injected during approximately the first 7 days, *i.e.* the greater part of the injected material." This does not amount to quantitative assay. In the absence of proof that hormones such as oestrin and the testis hormone are absorbed before the oily medium, and with the definite probability from our experiments that they are not, quantitative absorption cannot be assumed when oil is found in the subcutaneous tissues at autopsy, and quantitative absorption is the first essential in quantitative assay.

There are, however, two objections to the policy of postponing autopsy sufficiently long for the last injections to be absorbed: (*a*) the possibility cannot be excluded that some destruction of the hormone may take place during the prolonged incubation in oil at body temperature, and (*b*) if injections are given daily and each takes, say, four days to be absorbed, then the total amount absorbed by the animal will increase for the first 4 days and decrease in the 4 days after the last injection. Appreciable degeneration might take place in the stimulated organ when absorption fell below the threshold requirement some time during the last 4 days.

The results on rats and mice are not necessarily applicable to other experimental animals, and are almost certainly not true for the human subject. In the rat and mouse the facilities for subcutaneous absorption are very similar. A loose skin is connected with a thin body wall by a small amount of almost avascular connective tissue, and the conditions for absorption are bad. In other animals, such as the ferret, and particularly man, the skin and subcutaneous tissue are highly vascular, and conditions for absorption are far better. To judge by the absence of local reaction, the human subject can absorb 1.0 c.c. castor oil daily with comparative ease.

SUMMARY.

1. Olive, sesame and castor oils are absorbed very slowly after subcutaneous injection into rats and mice. Olive oil is slightly better than sesame, while castor oil is remarkably resistant to absorption.

2. Absorption is better in rats than in mice, but 0.1–0.2 c.c. of olive or sesame oil seems to be the maximum amount given as one injection which can be absorbed in a week. Larger amounts of sesame oil and even small amounts of castor oil appear to leave residues almost indefinitely.

3. Residual activity can be demonstrated in unabsorbed castor oil solution of oestrin as long as 15 days after injection into the ovariectomized mouse, by which time cornification has been induced and has passed off. This disposes of the suggestion that the hormone is absorbed rapidly and quantitatively in spite of the persistence of much of the medium.

4. Oils would thus appear to be highly unsuitable media for the injection of oestrin and similar hormones when quantitative assay is being attempted.

5. These results do not necessarily apply to animals other than rats and mice, in which the facilities for subcutaneous absorption are particularly bad.

We are much indebted to Dr G. F. Marrian for suggestions, and for discussion of the various points raised in this paper.

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THE EFFECT OF SYMPATHETIC NERVE STIMULATION ON THE POWER OF CONTRACTION OF SKELETAL MUSCLE.

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INTRODUCTORY.

IN recent years there have appeared from Orbeli's laboratory at Leningrad reports¹ of experiments which seem to show that stimulation of sympathetic nerves to skeletal muscle will partially relieve the fatigue that develops in such muscle when it is stimulated through its spinal nerves.

Orbeli's work has been repeated on various occasions with conflicting results. Wastl [1925] failed to confirm Orbeli's principal experiment; on frogs without circulation, sympathetic stimulation did not affect the muscle contractions, while on the cat with circulation intact the effect was often to depress the contractions, owing, no doubt, to the accompanying vaso-constriction. Negative results were reported also by Vazadse [1926]. Hunter [1925] reported that sympathectomized muscle in the seagull's wing fatigued more easily during flight than did the unoperated muscle. Coates and Tiegs [1928] found that indirectly stimulated sympathectomized muscle in the goat fatigued much more readily than did the control muscle; but the experiment was inconclusive, since possible injury to the muscle, owing to long deprivation of sympathetic nerves, was not excluded. Maibach's study [1928] on isolated frog muscle gave the first adequate confirmation of Orbeli's work. In the experiments of Baetjer [1930] on the cat with intact circulation, sympathetic stimulation often led to a marked increase in strength of contraction, while at other times, and under apparently similar conditions, it had a depressant effect, as Wastl has already found. Gede-wani [1930] has recently obtained Orbeli's effect on frog muscle.

¹ The publications of Orbeli and his co-workers have unfortunately not been available to us. The most recent account of them seems to be that of Brücke [1927].

A simple vascular explanation of the phenomenon has been excluded by the use of isolated muscle without circulation [Orbeli, Maibach]. Gedewani proposes a modified form of vascular interpretation that will be given special consideration below. Coates and Tiegs, Maibach and Baetjer ascribed their positive results to the action of a direct sympathetic supply to muscle. But the existence of a double innervation of striated muscle tissue in general has recently been seriously doubted. Boeke's own careful studies [1913, 1927] have been mainly on the mammalian eye and tongue musculature and do not necessarily apply to the limb muscle. The work of Agduhr [1920], indeed, affords evidence of their existence here, but Agduhr's work has been repeated with negative results by Hinsey [1927] on the cat, and by Coates and Tiegs [1931] on the dog; while Boeke's own experiments on the eye muscles have been called into question by Hines [1931], Wilkinson [1930] and Woollard [1931]. It seems, therefore, that a direct sympathetic innervation of striated muscle in general cannot yet be unreservedly accepted.

The case for such an innervation is even further weakened by the fact that in reptiles appropriate degeneration tests show that the unmyelinated nerves in muscle, which, following Kulchitsky [1924], we have been accustomed to look upon as sympathetic, are in reality somatic motor fibres [Tiegs, 1932]. In muscles in which the somatic nerves had degenerated, sympathetic nerves appeared on the blood vessels only, and, in analogy with Dale and Gaddum's work [1930] on the humoral origin of the Vulpian phenomenon, it was suggested that the Orbeli effect, if it really existed, might have a similar origin, and be due to the diffusion of an adrenaline-like substance from the vaso-constrictor nerves on to the adjacent muscle fibres.

Since Elliott [1904] suggested that sympathetic nerve action might be mediated by a peripheral liberation of adrenaline at the nerve endings, evidence for its occurrence has been forthcoming in the work of Loewy [1921] on an accelerator substance in the heart, and of Finkleman [1930] on an inhibitory substance in the gut; whilst recent experiments by Cannon and Bacq [1931] demonstrate the liberation at the pilo-motor endings in the cat, of a substance (sympathin) with adrenaline-like properties, which may pass into the blood and accelerate the denervated heart.

In the present paper we describe the results of experiments designed to test the validity of Orbeli's phenomenon, and the possibility of a humoral origin for it.

METHODS.

Frogs (*Hyla aurea*) have been used throughout.

The animals were decapitated, pithed, skinned, and eviscerated, and the front half was cut away. For the perfusion experiments the aorta was preserved. The gastrocnemius or sartorius was used for obtaining the fatigue records. These muscles may be stimulated through the sciatic plexus in the abdomen, or when required, through the eighth and ninth ventral roots. For the latter purpose the spinal column is carefully opened ventrally with small bone cutters, till the eighth and ninth nerves of the cauda equina are exposed. All dorsal roots are cut away. According to Langley and Orbeli [1910], the seventh nerve is the last nerve to convey preganglionic fibres to the sympathetic. Hence, electrodes applied to the sympathetic at or below the seventh ganglion should suffice to stimulate all the sympathetic fibres to the limb. The dissection of the sympathetic is performed with scrupulous care under a binocular lens. From the level of the sixth to the eighth spinal nerves it is freed from connective tissue, and usually from the sixth and seventh nerves, to which it adheres.

In all the experiments a constant frequency of stimulation of the muscle was maintained, namely, fourteen to the minute. Fine platinum-tipped electrodes were used; in the experiments involving direct stimulation of the muscle we employed fine flexible chrome-nickel wire, which could readily be bent round the muscle. Unless otherwise stated, maximal break shocks were used, derived from a small cored induction coil, fed from a 4-volt accumulator. The make-and-break apparatus, designed by Brown and Lees [1931], and previously tested out by us on the string galvanometer for uniform strength of break shock, gave satisfactory service. For stimulating the sympathetic, we have used a faradic current from a separate induction coil supplied by two dry cells, the secondary coil distance being so adjusted that there were no perceptible effects of current spread on to the neighbouring spinal nerves.

In recording the muscle contractions we have used an isotonic lever, without after-loading and suitably weighted, the resultant tension on the muscle being usually about 20 g.

For the perfusion experiments a cannula was made from a piece of glass tubing 8 in. long, drawn out at one end into a bent capillary. When the capillary end is inserted along the aorta, a steady perfusion usually flows with a head of about 5 to 6 in. or even less of Ringer fluid in the main tube. Sometimes gentle massage of the limb muscles is necessary

to start the flow. Oedema never occurs. The efficiency of the perfusion may be observed by the rate of exit of the perfusate from the abdominal vein, the renal portal vessels having previously been ligated. Frog Ringer has been used throughout for the perfusion; it was buffered with phosphates, and, unless otherwise stated, adjusted to pH 7.4.

RESULTS.

(a) *The effect of sympathetic nerve stimulation on the size of the muscle twitch before and during fatigue.*

(i) *Indirect stimulation.*

The experiments were made on the gastrocnemius and sartorius. The non-perfused frog, with ventral roots and sympathetic prepared, was pinned on its back in a paraffin-lined dish, and the dish tilted so that the muscle was under Ringer, leaving the nerves exposed. The tendon of the muscle was freed and attached by thread to the isotonic lever. Electrodes were placed respectively under the sympathetic and under the ventral roots and fastened in position. The coil distances for ventral root and sympathetic stimulation were adjusted, as already described, and thereafter left unchanged.

The experiment consists in recording on a slowly rotating drum the response of the muscle to rhythmical maximal break shocks, fourteen to the minute, applied to the ventral roots, and of observing the effect on the contractions of prolonged sympathetic stimulation at various intervals along the fatigue tracing.

A complete record of such an experiment is shown in Fig. 1. Sympathetic stimulation of 1 min. duration early in the contraction series is followed after a latent period of 10-15 sec. by an increase in strength of contraction, but the effect does not attain its maximum until half-a-minute after the cessation of stimulation, after which it gradually subsides, outlasting the period of stimulation by about 5 min. When the sympathetic is again stimulated for 1 min. the effect recurs, though with a noticeably longer latent period, and for a shorter duration. The four succeeding stimulations, of rather longer duration, are each followed by responses in which there is a progressive increase in the latent period and a corresponding decrease in the magnitude of the response and of its duration.

Numerous similar experiments on both gastrocnemius and sartorius enable us to make the following general statements:

(a) Very frequently, even when all conceivable precautions have been taken in making the preparation, sympathetic stimulation is com-

pletely without effect. The reason for its frequent absence has not been wholly elucidated. We cannot confirm the statement of Bouman [1931] that submaximal stimulation is a necessary condition, for in our own experiments we have invariably used maximal stimuli. One possible common source of failure is that the strength of stimulus that it is possible to apply to the sympathetic, without spreading on to the closely adjacent motor nerve, may not suffice to stimulate the sympathetic; for the stimulation threshold is stated to be much higher for the sympathetic than for the motor nerve. A second, more definitely proved deterrent,

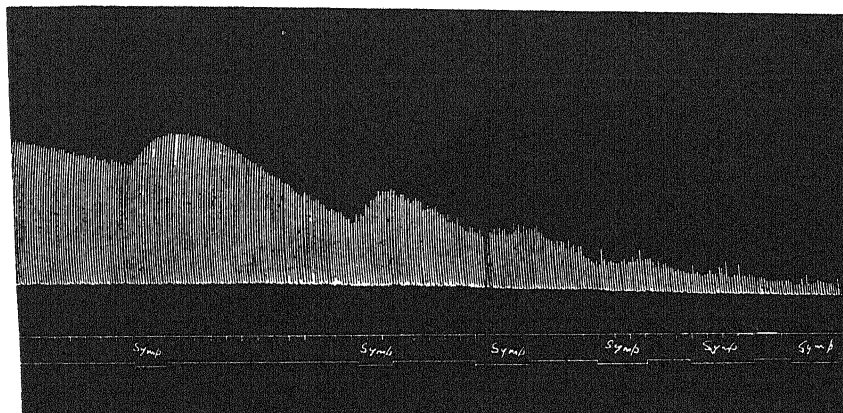


Fig. 1. Complete fatigue record of unperfused gastrocnemius stimulated with maximal break shocks through eighth and ninth ventral roots, rate fourteen per minute; showing effects of periodic prolonged sympathetic stimulation. Upper line, time in half minutes; lower line, sympathetic stimulation.

is low temperature. In our experience, with the temperature below about 12°C ., the Orbeli effect does not occur. (See further below: Action of adrenaline.)

(b) Sometimes the improvement in the contraction is only very slight, though even then the effect may long outlast the stimulation. Mild effects may consist merely in a temporary arrest in the rate of fatigue, and not in an actual improvement of contraction.

(c) When the effect is a marked one, it can be obtained on the same preparation several times in succession. This has never failed. We have one record from the sartorius when it appeared eight times in succession.

In fresh muscle Orbeli was unable to obtain the phenomenon, and concluded that the effect of sympathetic stimulation was to relieve fatigue and not to improve the contractions of unfatigued muscle.

Maibach [1928] confirms this. In our experience, sympathetic stimulation at the commencement of a fatigue tracing sometimes already gives a very mild effect; though for a later stimulation, at a time when fatigue has set in, the effect is very much greater. Since freshly prepared muscles are no doubt often in a partly fatigued condition, the observation does not conflict with Orbeli's view.

Conclusive evidence was sought in the following experiment. The preparation was set up as before, but in addition perfused through the aorta with well-oxygenated Ringer for 20 min., by which time any accumulated fatigue products should be removed. The perfusion fluid was now changed to non-oxygenated Ringer, ventral root stimulation

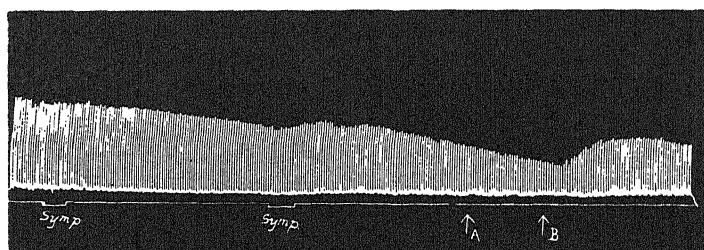


Fig. 2. Fatigue record of perfused gastrocnemius stimulated with maximal break shocks through eighth and ninth ventral roots, rate fourteen per minute; showing effect of sympathetic stimulation on the fresh and fatiguing muscle. 5 in. head of perfusion pressure increased at A to 8 in. Objective evidence for efficient perfusion is given by increased strength of contraction when perfusion fluid is replaced at B by adrenaline 1 : 1 million at 5 in. pressure head.

commenced, and the contractions recorded (Fig. 2). In the early contractions of the fresh muscle, it is evident, sympathetic stimulation for 1 min. is without perceptible effect. The electrodes are left in position. When partial fatigue has set in the sympathetic is stimulated again, and for an equal duration. On this second occasion there ensues a characteristic Orbeli response. It is evident, then, that the effect can only be obtained in fatigued muscle. (There are other features in this tracing that will be referred to below.)

(ii) *Direct stimulation.*

Orbeli reported that the improvement in strength of contraction occurred only in indirectly stimulated muscle, and suggested that the myoneural junction was directly concerned in the phenomenon. Michol [1930] claims that in the directly stimulated curarized gastrocnemius,

sympathetic stimulation is followed by an improvement in the strength of contraction, and urges that, in the Orbeli phenomenon, the sympathetic nerves exert their effect directly on the muscle tissue.

Our experiments were made on a batch of thirteen large summer frogs. The curare (Merck's) was kindly given to us by Prof. Osborne of Melbourne University. On the directly stimulated perfused or unperfused gastrocnemius, whether the curarization has been light or heavy, sympathetic stimulation is, in our experience, without perceptible effect on the strength of contraction of the fatiguing muscle. It seems doubtful whether the use of the highly purified curare (Boehm's), which Michol had at his disposal, could have altered the result.

Experiments undertaken on the non-curarized sartorius confirm Orbeli's view. The tibial end of this muscle is free from motor nerves. Hence, by applying the electrodes to the nerve-free end of the sartorius, the muscle may be directly stimulated to fatigue without involving indirect stimulation through any of the intramuscular nerves. In numerous experiments we find that the contractions thus elicited are not perceptibly affected by sympathetic nerve stimulation. But the uncertainty with which the Orbeli effect can be obtained, even in indirectly stimulated muscle, might be urged against the significance of this negative result. We have, therefore, sought for critical evidence by modifying the experiment in the following manner.

Ventral roots and sympathetic are prepared, and the preparation is set up without perfusion in the usual way. One pair of electrodes from the make-and-break apparatus is fixed under the ventral roots, a second to the nerve-free end of the sartorius. A commutator is inserted into the circuit in such a way that the break shock may be delivered alternately to the muscle and to its nerves throughout the whole fatigue series. Coil distances for maximal direct and indirect break shocks are determined. Since an interval of 4 sec. elapses between the individual break shocks, the quick sliding of the secondary coil to and fro to adjust for maximal direct and indirect stimulus does not present any difficulty. Once the two coil distances have been determined they are kept constant for alternate direct and indirect stimuli respectively throughout the whole fatigue tracing. When fatigue occurs the sympathetic is given prolonged stimulation, and its effect on the two sets of contractions observed.

Fig. 3 is a record from such an experiment. Alternating with the directly excited contractions are the indirectly excited ones, rather smaller in size, for fatigue has already partially set in. When the sympathetic is stimulated for a period of 90 sec., there occurs a gradual

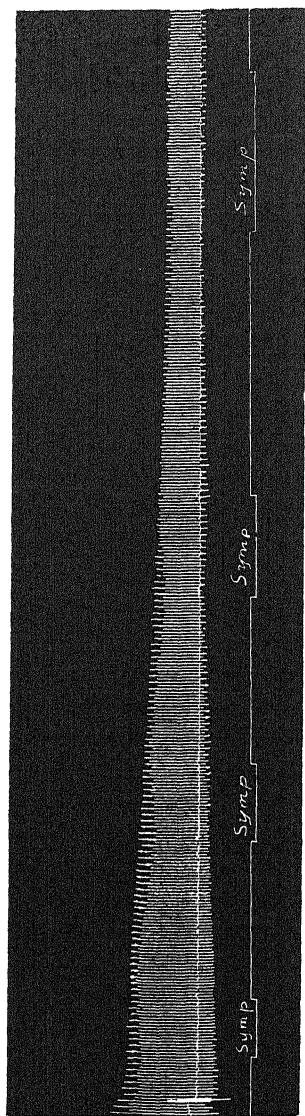


Fig. 3. Fatigue record of unperfused sartorius stimulated with maximal break shocks alternately directly and indirectly, rate fourteen per minute; showing selective effect of sympathetic stimulation on the indirectly excited contraction.

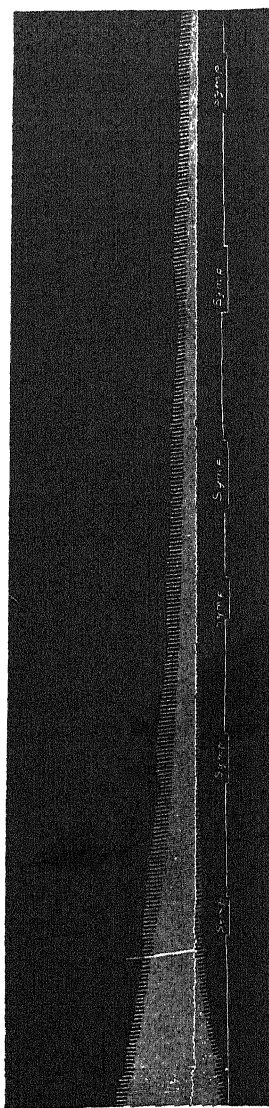


Fig. 4. As in Fig. 3, except that direct stimulation is submaximal, indirect stimulation maximal.

increase in the size of the indirectly excited contractions, attaining a maximum about 30 sec. after cessation of stimulation, and then gradually subsiding. This may be repeated a second, third and fourth time. In each case the effect is strictly confined to the indirectly stimulated contractions.

In view of a recent statement by Bouman [1931] that submaximal stimulation is a necessary condition for obtaining Orbeli's effect, we have modified the procedure by employing submaximal break shocks for the direct stimulation, maximal for the indirect, the indirectly excited contraction being, therefore, more powerful than the directly excited (Fig. 4). Nevertheless, for six successive stimulations of the sympathetic, characteristic Orbeli responses, confined to the indirectly excited twitches, are obtained. It must be concluded then, with Orbeli, that directly stimulated fatigued muscle will not respond to sympathetic stimulation.

(iii) *The possibility of artefact.*

It has been necessary to consider carefully whether, after all, the whole phenomenon may not be an artefact, or, at the best, some unsuspected vascular phenomenon. Obvious effects due to spread of current from the electrodes on the sympathetic are excluded by the fact that sympathetic stimulation and improvement in contraction do not synchronize, there being a variable latent period and a very long after-effect; and in any case the effects of deliberate spread of current are easily recognized. If the spread is great enough, the muscle passes at once into tetanus, which only momentarily outlasts the period of stimulation; while the muscle is undergoing relaxation from the tetanus, contractions of rather large size occur, but with the attainment of complete relaxation, regain their previous size. Occasionally another spread effect is seen, some of the contractions suddenly becoming larger; but the effect never outlasts the stimulation. A possible explanation for this is that the spread current, itself ineffective, reinforces the rhythmical stimulation on the ventral roots, giving rise to occasional "supramaximal" contractions.

That the Orbeli phenomenon is not the consequence of the faradic stimulation interfering in some obscure way with the ventral root stimulation, but that it is, on the contrary, due to the activity of living sympathetic nerves, seems clear from the following considerations:

(a) The progressive increase in latent period and weakening of the effect with successive sympathetic stimulations suggest rather the fatigue of some peripheral mechanism.

- (b) It is paralysed by ergotoxine. (See below: Action of ergotoxine.)
- (c) It may be reproduced, even in its details, by appropriate treatment with adrenaline. (See below: Action of adrenaline.)
- (d) The effect may be obtained only with the living sympathetic nerves.

The evidence for the last statement may be given here. It rests on the fact that crushing the sympathetic nerve distal to the seat of stimulation will prevent the onset of the Orbeli effect at a time when it would otherwise have occurred. Although in many preparations the Orbeli effect is unaccountably absent, yet, as already stated, if a strong effect is obtained once, it will occur without fail for several times in succession in that same preparation. It has been our aim to examine the

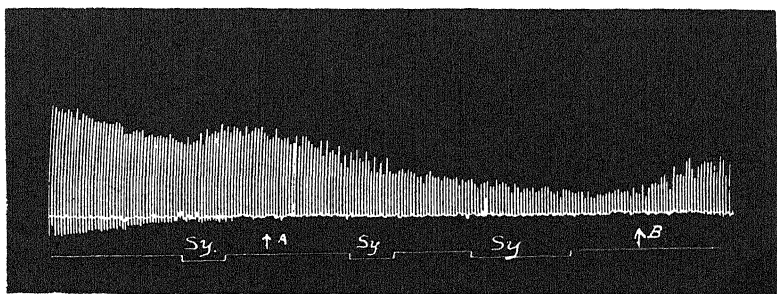


Fig. 5. Fatigue record of unperfused sartorius; maximal ventral root stimulation, rate fourteen per minute. Crushing of sympathetic nerve at *A* annuls any further responses of the muscle to sympathetic stimulation. At *B* adrenaline 1 : 100,000 applied directly to muscle.

effect of crushing the sympathetic nerve in such a preparation. Fig. 5 is a record of such an experiment. At *A* the sympathetic just below the electrodes is crushed with fine forceps. Although, previous to this, stimulation of the sympathetic has produced a strong rise in the contractions, stimulation of equal or much longer duration is now without effect. On the other hand, at *B*, direct application of adrenaline (1:100,000) to the surface of the muscle (sartorius) still produces a characteristic effect. It is evident that an uninjured sympathetic is a condition for the Orbeli response.

(iv) *The possibility of vascular origin.*

A simple vascular explanation is excluded by the fact that the effect is obtainable in isolated muscle. An alternative explanation, to meet the case of isolated muscle, is offered by Gedewani [1930]. Restoration of

circulation to a muscle in which it has been temporarily occluded will improve the strength of contraction in that muscle; and it is urged that in isolated muscle, stimulation of the vaso-constrictor nerves, by forcing stagnant blood from the larger into the smaller vessels, will produce a similar effect. But it is clear that such an explanation would not apply to a bloodless muscle well perfused with Ringer fluid. Yet in such muscle the characteristic Orbeli effect occurs (Fig. 2). It might be urged that sympathetic stimulation in some way actually improves the perfusion. But, in our experience, increase in the rate of perfusion, once an efficient perfusion is established, is without effect. For instance, in the experiment of which Fig. 2 is a record, the perfusion ran steadily under a 5 in. head of pressure. At *A* the pressure was increased to 8 in., without improvement in the contractions. At *B* the fluid in the perfusion tube was replaced by adrenaline 1:1,000,000 at 5 in. head of pressure; the powerful increase in the contraction may be taken as objective evidence for efficient perfusion. Whatever, then, the cause of the improvement of the contractions may be, it cannot be due to altered blood supply.

(v) *Discussion.*

Since sympathetic stimulation is able to improve the contractions only of indirectly excited muscle, it seems probable that its action is exerted somewhere on the nerve-muscle junction. Such a conclusion would be unavoidable if it were certain that events occurring in directly and indirectly stimulated muscle were identical. Myothermal experiments by Hartree [1929] suggest that this is, in fact, the case. In our own experiments there appears a slight but constant difference in the effects of direct and indirect stimulation. It will be seen (Figs. 3, 7) that, in the markedly fatigued muscle, for indirectly excited twitches the lever regularly "overshoots" the base line, but that this is much less apparent for the directly stimulated muscle, even for twitches of equal height. It is evident that in our experiments the directly stimulated muscle relaxes more slowly. This would seem to have its origin in the fact that, with the strong direct stimulus that it is necessary to employ for maximal contraction of a thick muscle, some of the superficial fibres pass, sometimes even visibly, into brief tetanus and so retard the relaxation. The conclusion, therefore, that directly and indirectly excited contractions are similar, and hence that sympathetic stimulation exerts its effect through the motor end-organ, does not seem to be invalidated. The remote possibility that sympathetic stimulation exerts its effects directly on the muscle tissue by counteracting fatigue products formed

only during indirect stimulation is evidently excluded by a record such as Fig. 3, where directly stimulated muscle in the midst of indirectly stimulated contractions fails to respond to sympathetic stimulation.

Turning now to the causation of the Orbeli effect, it is evident that it displays features that are reconcilable, though not exclusively so, with a theory of humoral origin. The rather long latent period would be the time that elapsed for the chemical factor to reach an effective concentration at its site of action. The delay in the attainment of maximum effect up to about 30 sec. after cessation of stimulus, suggests a diffusion of the chemical factor through an appreciable distance intervening between the site of its formation and of its action. The succeeding slow decline of the effect, with complete extinction after about 5 min., would be ascribed to a falling off in the concentration of the chemical factor at its site of action to below its effective value, due either to its destruction, or to its gradual dispersal through the tissue. The progressive weakening of the effect over a series of successive stimulations implies a formation of the chemical substance in smaller and smaller quantity, either owing to exhaustion of the supply of the precursor, or to a fatigue of the end-organ. The increase in the latent period that accompanies this progressive weakening of the effect is of particular interest. It is a necessary condition for the validity of the humoral theory, and would owe its existence to a slower and slower attainment by the chemical factor to effective concentration as it was produced in smaller and smaller quantity.

It must be noted at the same time that even if we adopt the theory of a chemical substance mediating sympathetic nerve action, this in itself will not exclude a direct sympathetic innervation of muscle if the physiological evidence suggests it. But, in the present case, it would seem that, if the physiological evidence be taken to indicate a sympathetic innervation other than that of the blood vessels of the muscle, then, to be consistent, we should seek those sympathetic endings in the motor end-organs themselves and not on the muscle fibre. Now it is a significant fact that, of all the nerve endings in muscle that histologists have regarded as sympathetic, hardly any have ever been described in the motor end-organ itself.

(b) The action of adrenaline.

In a humoral theory of peripheral sympathetic nerve action one would naturally look to adrenaline as a possible mediator between the nerve endings and the receptive material of the effector cell. For this

reason it has been necessary to examine once again the action of adrenaline on striated muscle.

The literature on this matter is conflicting. Physiologically strong doses are said to act as depressants [Boruttau, 1899; Takayasu, 1916]. Beneficial effects on muscle, first reported by Oliver and Schäfer [1895], were later ascribed to impurities in the preparation [Schäfer, 1916]. Hess and Neergaard [1924] found adrenaline to be without perceptible effect on the contraction of fresh frog muscle. Dessy and Grandis [1904] observed marked recovery of the fatigued frog's gastrocnemius when adrenaline was injected into the lymph sac. Gruber [1914a] obtained this effect on directly stimulated denervated muscle of the cat with circulation intact, and argued for a direct action of adrenaline on the muscle tissue. Guglielmetti [1922], on the contrary, found an effect only with indirectly stimulated muscle, and suggested an action on the motor end-organ. Wastl [1925] obtained no effect on the directly stimulated frog's sartorius; while in indirectly excited mammalian muscle with circulation intact, adrenaline had usually a powerful depressant effect, due to vaso-constriction [Wastl, 1928].

Our experience with the Orbeli phenomenon suggests an explanation for some of these discordant observations. The positive results of Dessy and Grandis and of Guglielmetti were from indirectly stimulated fatigued muscle, whereas it was in experiments on unfatigued or directly stimulated muscle that adrenaline was without effect. Some of the discordant results on mammals (Wastl) may possibly be due to altered blood supply, which has a much more profound effect on mammalian muscle than on that of the frog.

(i) *Action of adrenaline on indirectly stimulated muscle.*

In our initial experiments the adrenaline was simply added to the fluid in which the muscle was bathed. This frequently leads to strong recovery of a muscle fatigued through its nerve (*e.g.* Fig. 5). The effect, however, is inconstant, especially with the thick gastrocnemius, and is of interest only in that it excludes a possible vascular explanation.

The later experiments were all made on the perfused preparation. The procedure consists simply in replacing the Ringer fluid in the perfusion cannula with adrenaline of known concentration. Parke, Davis & Co.'s or Ciba adrenaline was used throughout, the 0.1 p.c. solution being diluted with frog's Ringer as required immediately before use.

When adrenaline 1:1 million is perfused into the fatigued muscle it usually brings about an improvement in strength of contraction. The

magnitude of the effect varies greatly. Sometimes the recovery is small, but at other times it may be so powerful that a muscle, which has become well-nigh exhausted, is restored to the condition of a fresh muscle (Fig. 6). Since severe vaso-constriction with complete stoppage of perfusion soon appears, a vascular interpretation is obviously excluded.

The strongest effects are obtained only if the temperature is about 16–20° C., and if fatigue has set in fairly rapidly; but even under these circumstances only mild effects are sometimes obtained. The cause for this variation has not been discovered. We suspect that the “condition” of the animals may in some way be involved. For example, in a series of twenty-four frogs, which were deliberately held in captivity in a dark

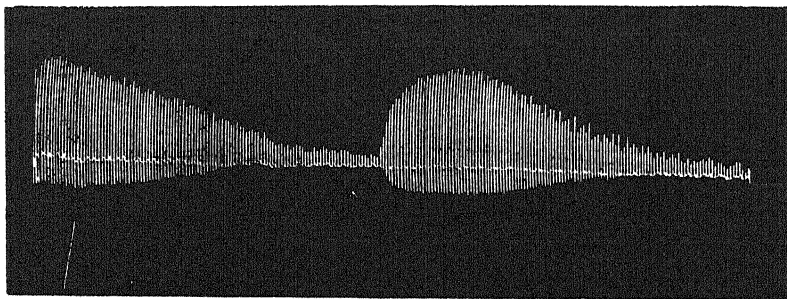


Fig. 6. Gastrocnemius, perfused; maximal ventral root stimulation, rate fourteen per minute; showing an exceptionally powerful response of the muscle to adrenaline 1 : 1 million perfused into it.

cool tank for 3 months, and which were obviously ill-conditioned, ten failed to give any response to adrenaline, in six the effect was very mild, while in eight it was strong. Contrasted with this, in a series of thirty-two freshly captured frogs, six failed to respond; in the remaining twenty-six the response was pronounced.

Undue exposure to heat also renders the frogs insensitive to adrenaline. For instance, in a batch of fourteen frogs that had been kept for about 6 hours at 26° C., only two would respond to adrenaline, and then only feebly, even when the adrenaline was applied at a temperature of 18° C.

In our experience the contraction of unfatigued muscle is never improved by adrenaline; and although a fatigued muscle may occasionally be restored to the condition of the fresh tissue, improvement beyond that does not occur.

(ii) *Action of adrenaline on directly stimulated muscle.*

Contrary to Bouman, we find that adrenaline has a profound reviving effect on the frog's gastrocnemius when fatigued through electrodes applied directly to the muscle; but as indirect stimulation of the muscle through its intramuscular nerves is not excluded, the experiment has little interest.

When direct stimulation is ensured by previous curarization, adrenaline is without visible effect. This conclusion is based on numerous experiments, and confirms the analogous result with sympathetic stimulation.

The effect of adrenaline on directly stimulated uncurarized muscle we have examined by the use of the sartorius stimulated to fatigue

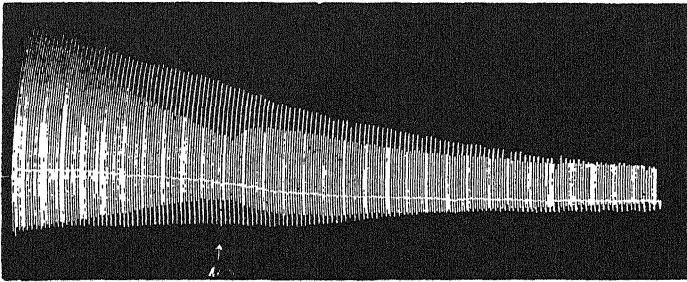


Fig. 7. Sartorius, perfused; maximal alternate direct and indirect stimulation, rate fourteen per minute; showing selective effect on the indirectly excited contractions of perfusing adrenaline 1 : 1 million into the muscle.

through its nerve-free end. In numerous experiments we find that adrenaline is without perceptible effect.

Critical objective evidence may be obtained by the method of alternate direct and indirect stimulation already employed for examining the effect of the sympathetic on directly excited muscle. Fig. 7 shows a record from such an experiment. It is evident that, when adrenaline is perfused into the fatiguing muscle, it affects only the indirectly excited contractions.

These observations are in contradiction to those of Gruber [1914 a], who apparently demonstrated a direct action of adrenaline on the muscle tissue in the cat. In Gruber's experiments stimulation of the muscle through its intramuscular nerves was excluded by previous denervation. It is desirable then to consider the possibility that denervation may have sensitized the muscle to adrenaline. For the purpose we have, in a series

of twenty frogs, cut the sciatic nerve just above the knee and allowed it to degenerate for periods varying from 44 to 61 days. Despite the prolonged inactivity of such a gastrocnemius, it yields a normal fatigue tracing with direct stimulation. In our experience adrenaline 1:1 million perfused into such muscle at the onset of fatigue is without perceptible effect on the contraction. For purposes of control the opposite gastrocnemius has been simultaneously stimulated through its nerve, and its contractions recorded along with those of the operated muscle on the same drum. In seventeen of the twenty frogs, in all of which the operated gastrocnemius remained unaffected, the unoperated muscle responded to

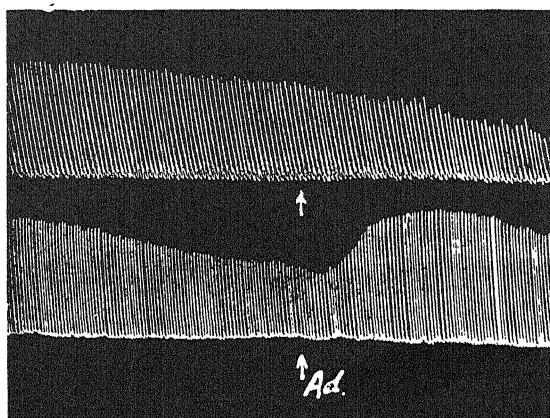


Fig. 8. Simultaneous records of contractions of denervated gastrocnemius (above) and of the normal opposite control gastrocnemius (below). Indirect stimulation of latter. Adrenaline 1:1 million, perfused into both muscles through aorta, affects only the normal muscle. Only the end portion of fatigue record shown.

the perfused adrenaline in the usual manner (Fig. 8). We cannot reconcile these results with Gruber's observation. He ascribed his results to a partial restoration of the stimulation threshold of the muscle to its value for the unfatigued muscle. We had hoped, therefore, to obtain Gruber's effect by changing to submaximal stimulation at the onset of fatigue, for a submaximal stimulus might be expected to become more effective when the stimulation threshold was lowered by adrenaline. But adrenaline proved to be as ineffective with submaximal stimulation as with maximal. Only a few experiments were undertaken to estimate the excitability of the muscle before and after adrenaline. Secondary coil distance at which just perceptible contraction of the muscle occurred, was used as a criterion for threshold stimulation. In the few experiments

that we have undertaken on the denervated muscle, the increase in threshold as fatigue set in was not lowered by perfusion of adrenaline into the muscle.

From these observations it seems legitimate to infer that, whatever direct action adrenaline may have on the muscle tissue, the observed beneficial effect that it has on indirectly stimulated muscle is not the outcome of such action, but that the adrenaline exerts its effect either on the intra-muscular nerves or on the nerve end-organs. The former possibility may apparently be excluded by applying adrenaline to the motor nerve before it enters the muscle, when it is, in our experience, without effect. We must conclude then, with Guglielmetti, that adrenaline acts somewhere on the junctional tissue.

(iii) *Effect of iodo-acetic acid.*

Before its apparent action on the motor end-organs was found, we suspected that the reviving effect of adrenaline might be a manifestation of its well-known acceleration of lactic acid formation from glycogen. To examine this further, we tested the action of adrenaline on muscle poisoned with iodo-acetic acid. For the purpose the hind limb preparation was placed for 30 min. into well-oxygenated Ringer fluid containing 1/25,000 neutralized iodo-acetic acid. The iodo-acetic acid was prepared from Merck's monochloroacetic acid, according to Abderhalden and Guggenheim's [1908] modification of Victor Meyer's method. Before use it was neutralized to pH 7.4 with NaOH. The peculiar type of fatigue tracing that such muscle now yields [Lundsgaard, 1930] is in our experience unaffected by perfusion of adrenaline into the muscle. The interpretation of this must for the present remain obscure.

(iv) *Effective concentration of adrenaline.*

In experiments with adrenaline we are continually struck by the extraordinary variation in sensitiveness of the muscle to this substance. For while, in a batch of freshly caught animals, some react powerfully, in others the response is weak, or at times even absent. In our experience adrenaline 1:1 million will suffice to give the maximum effect that any particular muscle is capable of; at any rate, a weak response to adrenaline at this concentration is not increased by stronger adrenaline.

Experiments to determine the highest dilution of adrenaline to which the perfused muscle (gastrocnemius) can respond were undertaken on a batch of thirty-five freshly caught summer frogs at temperatures between 19–22° C. Stimulation was usually done through ventral roots

rather than through the common nerve. The experiment consists simply in replacing the perfusion fluid with adrenaline at known concentration. We find that, while some muscles respond only weakly, or even not at all, with adrenaline 1:1 million, others respond well to 1:5 million, and, while most failed to respond to 1:10 million, we have one case where a muscle gave a strong response to adrenaline 1:20 million. It is evident that, in the presence of this extraordinary variability, some muscles may respond to adrenaline at even much higher dilution. But we have no record yet where this has, in fact, occurred.

It should be added that vaso-dilation in response to low concentration adrenaline is not the cause of this improvement; for deliberate increase in efficiency of perfusion is without such effect (Fig. 2).

(v) *Effect of hydrogen-ion concentration.*

Only a small number of experiments were undertaken to examine the effect of varying hydrogen-ion concentration on the adrenaline response. In our experience strong effects are obtainable when adrenaline is perfused into the muscle in Ringer fluid over a range of pH from 8.1 to 5.9; and adrenaline 1:20 million has given responses at pH 8.1, 6.6, 5.9 quite as strong as that obtained at pH 7.4. Whether the minimum concentration of adrenaline to which any muscle can respond is altered by the hydrogen-ion concentration of the perfusion fluid, we have not determined.

(vi) *The effect of temperature.*

The observations hitherto recorded were made in warm weather at a room temperature of at least 17° C. Later, during the winter months, when we had occasion to repeat some of these experiments, we were surprised to find adrenaline without, or almost without, effect. The possibility that winter frogs, perhaps owing to low "condition," were insensitive to adrenaline, was easily disproved by repeating the experiment in a warm room, when adrenaline had the usual effect.

Examination of the matter showed that the inhibitory factor was low temperature. With the temperature below about 8° C., adrenaline 1:1 million is almost always without effect, and at the best, only very mild responses are obtained. Between about 8–15° C., effects are more numerous and sometimes even strong, but the most powerful responses are obtained only with the temperature between about 16° and 20° C. This unexpected effect of low temperature was later confirmed on a batch of twenty freshly captured summer frogs. In nineteen of the

twenty frogs the gastrocnemius, contracting in Ringer cooled to between 3–5° C., failed to respond to adrenaline 1 : 1 million perfused into it; in the one exception a very mild effect occurred. In two other cases tested, even the massive dose 1 : 100,000 was without effect. It is worth recording that the usual powerful vaso-constrictor action of adrenaline was also practically absent.

It was these observations with adrenaline that led us to examine the effect of cold on the occurrence of the Orbeli phenomenon. As already stated the phenomenon does not occur, in our experience, at low temperatures. This statement is based on observations made on a batch of twenty freshly captured winter frogs at temperatures between 8° and 12° C., and later on a batch of ten freshly captured summer frogs in which the muscle was contracting in Ringer cooled to about 5° C. All the experiments were made on the perfused preparation, and the perfusion fluid replaced at the end of each experiment with 1 : 1 million adrenaline at the same temperature. In all cases sympathetic stimulation was without effect, and even the adrenaline, at the best, had only a mild action. In the middle of winter in a warm room, at a temperature of 17° C., we have obtained powerful Orbeli responses, so that the variation is due to change of temperature and not of season.

(vii) *Repeated application of adrenaline.*

The close parallelism that exists between the effects on skeletal muscle of adrenaline and sympathetic nerve stimulation, seems at first sight to fail in one important detail. In our experience the adrenaline response is usually, though not always, much stronger than the Orbeli effect [*e.g.* Figs. 2, 5, 6]. Moreover, when once a strong adrenaline effect has subsided, it will not recur with further application of adrenaline of the same or higher concentration. (A mild effect in response to adrenaline at high dilution will not, however, prevent a subsequent even greater effect with stronger adrenaline.) But it is a characteristic of the Orbeli effect that it may be obtained several times in succession in the one preparation. It is important for the humoral theory that this discrepancy should be examined.

It seems likely that the difference is owing to the method of administering the adrenaline. Our hypothesis that the Orbeli effect may be caused by a vaso-constrictor substance (adrenaline?) diffusing from the sympathetic nerves of the blood vessels on to the striated muscle tissue, must have two consequences: (*a*) the chemical factor must be liberated at physiologically high concentration—sufficient to cause vaso-con-

striction; (b) it must be formed in relatively small quantity since the whole effect is not of very long duration. We have attempted to reproduce these conditions in the following way:

A perfusion preparation is set up in the usual manner. When fatigue in response to rhythmical stimulation of the sciatic plexus begins to appear, the Ringer fluid is removed from the perfusion tube and replaced by about 0.5 c.c. of 1 : 1 million adrenaline, which is slowly run into the preparation. The perfusion tube is then quickly refilled with Ringer, which is forced into the tissue at a pressure sufficient to overcome the vaso-constriction which has by this time set in. The vaso-constriction

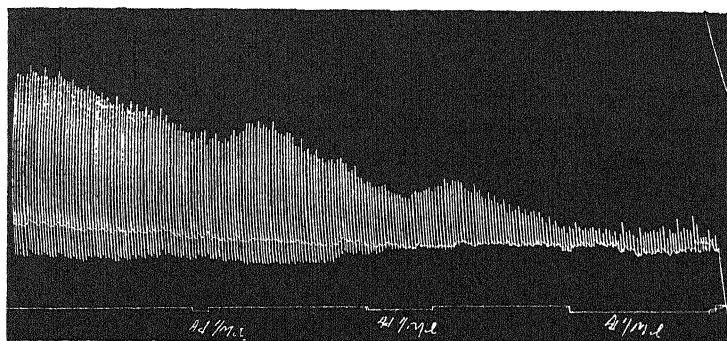


Fig. 9. Gastrocnemius, perfused; maximal indirect stimulation, rate fourteen per minute; showing effects of periodic perfusion of 0.5 c.c. adrenaline 1 : 1 million into the muscle. The signal indicates duration of the adrenaline in the adjustable part of the perfusion cannula, and not within the muscle itself. In the intervals between adrenaline perfusion, pure Ringer is forced through the muscle.

gradually declines again as the adrenaline is washed out of the vessels, till after about 5 min. it disappears. Meanwhile the muscle itself has begun to recover, but the effect is of short duration only, thus differing from the prolonged effects that one usually obtains with adrenaline when it is allowed to remain in the blood vessels. But when the operation is now repeated a second and a third time, the adrenaline response recurs (Fig. 9), and in this way the effect of repeated sympathetic stimulation is imitated.

(viii) *Discussion.*

From the foregoing it is evident that all the effects of sympathetic stimulation may be obtained also with adrenaline. This in itself does not of course prove that an adrenaline-like body mediates the action of

sympathetic nerves within the muscle. At the same time, no fact has been discovered that is at variance with such a view.

As with sympathetic stimulation, the site of action of adrenaline would seem to be the motor end-organ, for no effect has been obtained on directly excited contractions. Michol's claim that sympathetic stimulation can improve the contraction of curarized muscle has not been confirmed either with sympathetic stimulation or with adrenaline. Should it, however, ultimately prove to be correct, an explanation for it might be sought in the work of Gruber [1914 b], who has described an antagonism between curare and adrenaline on muscle excitability. This would then become a distinct argument in favour of adrenaline formation in the muscle, while at the same time the effect would be similar to the Orbeli effect in outward appearance only.

If we adopt the current form of the all-or-nothing law as applied to muscle, *i.e.* that the strength of contraction of a muscle fibre is independent of the strength of the effective stimulus, then the following explanation might be offered for the adrenaline and Orbeli effects. With onset of fatigue in a muscle stimulated through its nerve, there occurs not only a fatigue of the individual muscle fibres, but also a fatigue of the end-organs, some of which begin to fall out of action. As paralysis of the end-organs progresses, the indirectly excited contractions will fall off more rapidly than the directly excited. The effect of adrenaline (or of sympathetic stimulation) would appear to be to restore these endings to action, and hence the improvement of the contraction would be limited by the state of fatigue of the muscle fibres at the time. We have not yet undertaken experiments to test the validity of this explanation.

(c) *The action of ergotoxine.*

Ergotoxine, in sufficient concentration, produces "a specific paralysis of the motor elements in the structures, associated with sympathetic innervation, which adrenaline stimulates" [Dale]. From its use, therefore, additional evidence may be expected concerning the validity of the Orbeli effect. Orbeli himself reported that sympathetic stimulation was without effect after ergotoxine poisoning. Baetjer [1930], on the contrary, still obtained the characteristic improvement of contractions.

An experimental difficulty is met when this matter is examined on a series of frogs; for when the effect is obtained even after administration of ergotoxine, incomplete poisoning may be the cause; whereas a failure of the effect to appear may not necessarily imply that it has been annulled, since even without ergotoxine its occurrence cannot be relied on.

Critical evidence has, therefore, been sought from those preparations that have already yielded a good Orbeli response. The ventral-root sympathetic preparation is set up as usual and gently perfused with Ringer. Preparations that do not respond to sympathetic stimulation are discarded; but in those preparations that yield a good Orbeli effect the Ringer fluid is quickly replaced by ergotoxine 1 : 50,000 which is perfused for the remainder of the experiment into the muscle tissue. The electrodes on the sympathetic are left in position. As already stated, a preparation that initially responds well to sympathetic stimulation can be relied upon to react several times in succession. But in our experience sympathetic stimulation within 5 min. after perfusion with

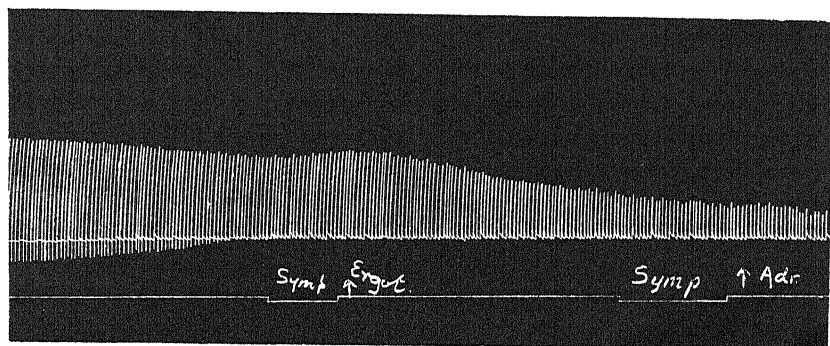


Fig. 10. Action of ergotoxine; gastrocnemius, perfused; maximal indirect stimulation, rate fourteen per minute. Perfusion of 1 : 50,000 ergotoxine prevents a second Orbeli response within 5 min. and an adrenaline (1 : 1 million) response within 8 min.

ergotoxine fails to give any further responses (Fig. 10). We may infer from this that the Orbeli effect has in fact been annulled by the ergotoxine.

Paralysis of the adrenaline effect could safely be examined without these precautions, since adrenaline in warm weather rarely fails to produce a strong response. In our experience, the response of a muscle to adrenaline 1 : 1 million is usually completely inhibited by ergotoxine 1 : 50,000 within about 6 to 10 min. (Fig. 10). To be doubly sure that a preparation was sensitive to adrenaline, we have tested the effect of ergotoxine in preparations that have already responded once to adrenaline, but in which the adrenaline had been completely washed out by subsequent perfusion with Ringer. (Disappearance of vaso-constriction was taken as a criterion for complete removal of adrenaline.) In such preparations we find that within about 6 to 7 min. ergotoxine 1 : 50,000

will annul the usual powerful response of the fatigued muscle to adrenaline 1 : 1 million.

It should be added here that the adrenaline response may sometimes still be obtained when the Orbeli effect is already inhibited; but this must not be taken as evidence against our hypothesis that liberation of an adrenaline-like body within the muscle underlies the Orbeli effect, for the higher concentration at which the adrenaline is artificially administered may well be the cause of the difference.

CONCLUSION.

In the foregoing account we have seen, in confirmation of Orbeli, that the fatigue of a skeletal muscle with indirect stimulation may be relieved by stimulating the sympathetic nerves to the muscle. We have seen also that improved blood supply is not the cause of the phenomenon.

One would most naturally ascribe the recovery of the muscle to the operation of its direct sympathetic nerve supply. But we have seen that the histological evidence for this double innervation of the muscle tissue is not beyond suspicion. The existence of the Orbeli effect might be regarded as physiological evidence in favour of the histological theory. But a serious discrepancy then appears. For, whereas the effects of sympathetic nerve stimulation are exerted apparently on the motor end-organ and not on the muscle fibre direct, most of the nerve fibres to which histologists have ascribed a sympathetic origin have no relation to the end-organ, but terminate independently on the muscle fibre. In the extreme view of Kulchitsky and of Hunter, they even end on wholly different muscle fibres.

It is evident then that the theory of double innervation in its present form is inadequate to explain Orbeli's phenomenon, and this is true especially on the quantitative side, for the recovery produced by sympathetic stimulation may sometimes be so pronounced (Fig. 1) that it would be necessary to assume a sympathetic innervation of a large proportion of the motor end-organs. Possibly with better histological methods it will eventually be possible to display such a nerve supply. But it seems fair comment to say that at present there is little to indicate its existence.

As an escape from this dilemma we may turn to the sympathetic nerves on blood vessels. Here they may regularly be displayed in large numbers. But an effect on the muscle tissue, produced by nerves that are not directly connected with it, implies a humoral mechanism for the effect. Now we have seen that the Orbeli effect presents features that

can most readily be reconciled with a theory of humoral origin. And, moreover, a known chemical substance, adrenaline, is able to reproduce even in detail all the effects that may be obtained by sympathetic nerve stimulation. If to this we add the evidence that is accumulating for the formation of an adrenaline-like substance at the endings of sympathetic nerves [Elliott, Loewy, Finkleman, Cannon and Bacq], then the case for a humoral origin of the Orbeli effect, which the histological evidence seems to force on us, becomes very strong.

At the same time, it is, of course, clear that the humoral theory, even if it had been conclusively demonstrated, would not necessarily have conflicted with the view of a direct sympathetic innervation of the motor end-organ, if the existence of such were directly demonstrated; for the action of such nerves might well be transmitted chemically. In fact, the only serious objection that can at present be brought against such a view, other than the lack of histological support for it, is the long delay in attainment of the maximal effect in the Orbeli response, usually ranging up to half-a-minute after cessation of stimulation; a delay which suggests that an appreciable distance intervenes between the site of formation of the chemical substance and its point of action.

It is evident that further histological investigation is desirable.

SUMMARY.

When the sympathetic nerves to isolated fatiguing skeletal muscle are stimulated, there occurs a marked recovery in strength of contraction in such muscle (frog). The effect is obtained only for indirectly excited contractions, never for directly excited. It seems, therefore, to be exerted through the motor end-organ. On these points our results confirm those of Orbeli.

The possibility of this effect being an experimental artefact is excluded. Its occurrence is not dependent on improved blood supply. Its features suggest a humoral mode of origin.

The effects of sympathetic stimulation may be imitated even in detail by appropriate treatment with adrenaline.

The bearing of these observations on the current histological theory of double innervation of muscle is discussed.

In conclusion we have much pleasure in acknowledging our indebtedness to Messrs A. Douth and H. Ennor for technical assistance.

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OBSERVATIONS ON THE PUMPING ACTION OF THE HEART.

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A STUDY of the factors which influence the systemic flow is complicated by the number involved and by their interaction. In general it may be stated that the pumping action of the heart and the state of the peripheral circulation are the chief factors determining the systemic flow. Starling and his co-workers [1912, 1914] laid down the principles which govern the pumping action of the heart in the heart-lung preparation, one of them being that a healthy heart maintains the venous pressure at zero for inflows up to 1 litre per min. It is desirable therefore to enquire whether the heart in the body reacts to all changes in the peripheral circulation by maintaining the venous pressure constant. The object of this paper is to describe experiments showing that the same order of change in the systemic flow is produced by occlusion of certain systemic blood vessels, whether the heart or a pump maintaining the venous pressure constant is the motive force for driving the blood through the circulation. The results of the investigations suggest that the heart in the body reacts to certain systemic circulation phenomena by adjusting its output so as to maintain the venous pressure constant, and in this respect therefore behaves like the heart of the heart-lung preparation.

I. DESCRIPTION OF PREPARATION IN WHICH A PUMP REPLACED THE HEART.

In the preparation about to be described the delivery of the pump was regulated so as to maintain the venous pressure as nearly constantly at zero as possible under all conditions. An electrically-driven Albany No. 0 rotary pump was found to fulfil this purpose admirably.

Dogs weighing approximately 10 kg. were used. Fig. 1 shows a diagram of the preparation.

Blood flowed from the s.v.c. (*B*) and the i.v.c. (*D*) to a small reservoir *F*. At the same time blood was drawn from the reservoir by the pump *G*. From the pump it passed through a heating spiral *H* and a mechanical stromuhr *J* [Barcroft, 1929]. The arterial blood-pressure was recorded by a mercury manometer attached at *K*. The temperature of the blood was taken at *L* and was maintained at 37° C. Finally the blood entered

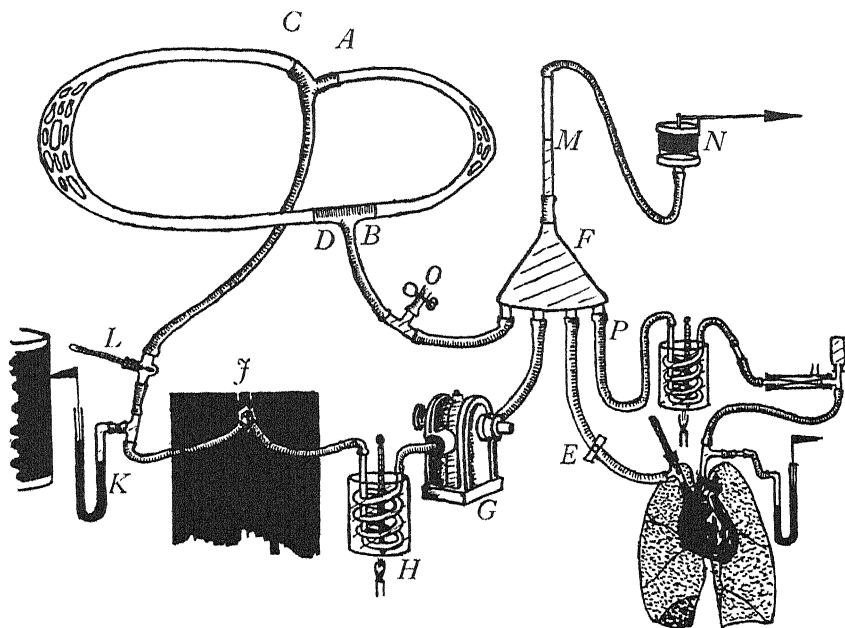


Fig. 1. Diagram of preparation in which a pump replaced the dog's heart. *A*, brachiocephalic artery; *B*, s.v.c.; *C*, thoracic aorta; *D*, i.v.c.; *O*, burette; *F*, blood reservoir; *M*, tube above reservoir for regulating venous blood-pressure; *N*, piston recorder; *E* and *P*, tubes to and from heart-lung preparation; *G*, pump; *H*, heating spiral; *J*, mechanical stromuhr; *K*, arterial blood-pressure manometer; *L*, thermometer.

the brachiocephalic artery at *A* and the thoracic aorta at *C*. The pressure of the blood in the reservoir *F* was measured by the height of the blood in the narrow tube *M*. The reservoir was placed so that a mark on the tube *M* represented the level of the disused heart in the animal. When the blood was at this level the venous pressure was therefore approximately zero. The output of the pump was regulated so as to maintain the blood level at the mark on the tube *M* under all conditions. A piston recorder *N* showed graphically whether the experimenter had been successful in keeping the blood level constant. At the beginning of the experiment

blood was added through the burette attached at *O* till the arterial blood-pressure was 80 mm. of mercury.

The arrangements for oxygenating the blood were as follows. Blood flowed from the reservoir *F* through the tube *E* to a heart-lung pre-

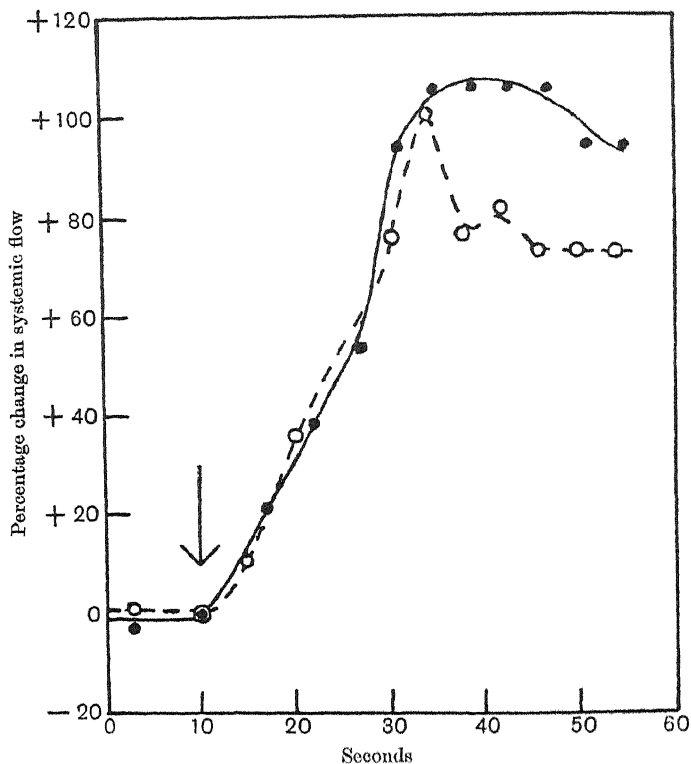


Fig. 2. Variations in the systemic flow in the animal and in the pump preparation after the injection of 50 c.c. blood. Continuous line: Animal. Initial flow, 530 c.c. per min. Broken line: Pump preparation. Initial flow, 440 c.c. per min. The injection was made at the time indicated by the arrow.

paration. Oxygenated blood from the heart-lung preparation returned to the reservoir *F* through the tube *P*. Thus oxygenated blood entered the reservoir *F* continuously and mixed with the blood returning from the animal. As the pressure in the reservoir *F* was maintained constant the flow to and from the heart-lung preparation was constant.

II. VARIATIONS IN THE SYSTEMIC FLOW IN THE ANIMAL AND IN THE PREPARATION IN WHICH THE PUMP REPLACED THE HEART.

A full account of the study of certain variations in the dog's systemic flow has been given in a previous paper [Barcroft, 1931]. Typical

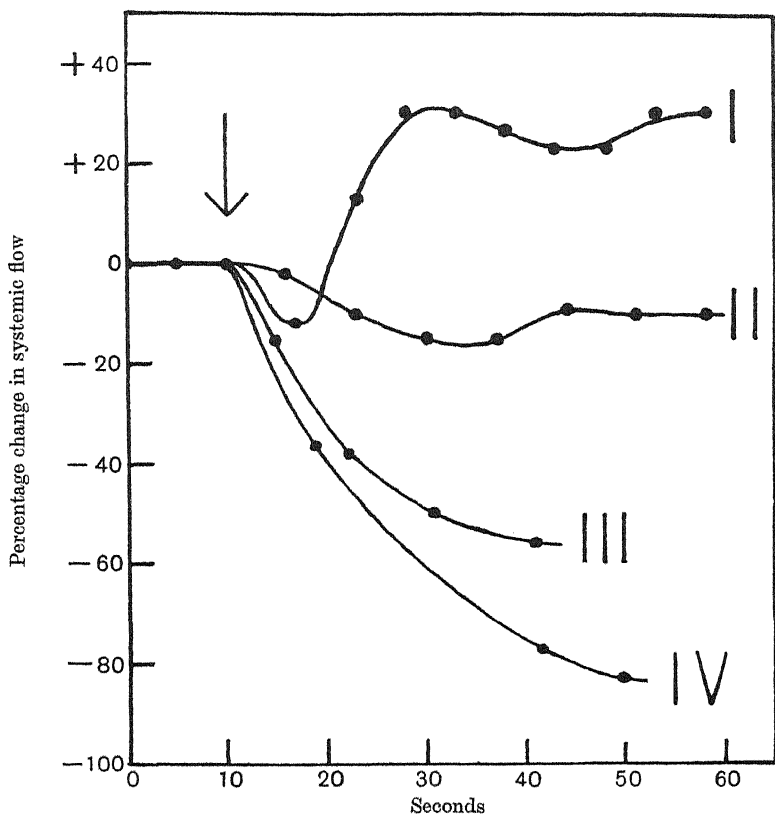


Fig. 3. Variations in the systemic flow in the animal after occlusion of a great vessel. Curve I: Occlusion of the thoracic aorta. Initial flow, 600 c.c. per min. Curve II: Occlusion of the brachiocephalic artery. Initial flow, 460 c.c. per min. Curve III: Occlusion of the s.v.c. Initial flow, 860 c.c. per min. Curve IV: Occlusion of the i.v.c. Initial flow, 680 c.c. per min. Each occlusion was made at the time indicated by the arrow.

examples are shown in the figures here. These variations were obtained in animals after section of the vagi, and after destruction of the brain.

Two experiments have been performed, using the preparation in which the pump replaced the heart. Uniform results have been obtained. Typical examples are shown.

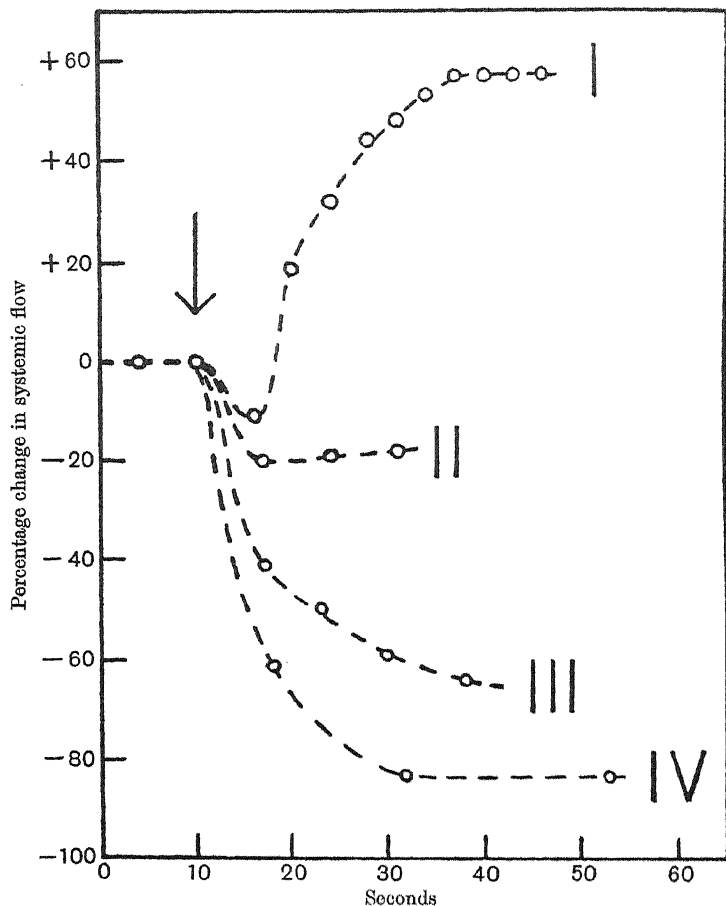


Fig. 4. Variations in the systemic flow in the pump preparation after occlusion of a great vessel. Curve I: Occlusion of the thoracic aorta. Initial flow, 620 c.c. per min. Curve II: Occlusion of the brachiocephalic artery. Initial flow, 540 c.c. per min. Curve III: Occlusion of the s.v.c. Initial flow, 560 c.c. per min. Curve IV: Occlusion of the i.v.c. Initial flow, 490 c.c. per min. Each occlusion was made at the time indicated by the arrow.

(1) *Injection of blood.*

Fig. 2 shows that 50 c.c. of blood injected into the vascular system increased the systemic flow in the animal and in the pump preparation.

In the animal the blood was added through a burette attached to the femoral vein. In the pump preparation it was added through a burette attached at *O* (Fig. 1).

(2) *Occlusion of one of the great vessels.*

Fig. 3 shows variations in the systemic flow in the animal after occlusion of one or other of the great vessels. Typically the systemic flow

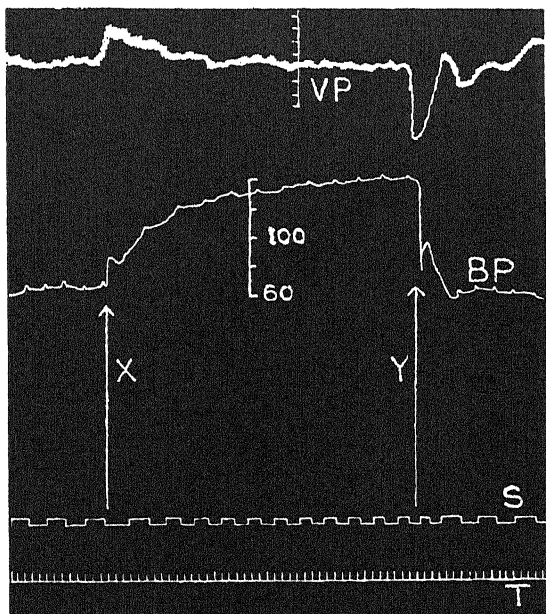


Fig. 5. Tracing taken during experiments using pump preparation. The thoracic aorta was occluded at *X* and released at *Y*. v.p. Venous blood-pressure. Scale cm. of water. B.P. Arterial blood-pressure. Scale mm. of mercury. S. Stromuhr, 26 c.c. of blood passed through the instrument between successive strokes. T. Time in seconds.

was increased by occlusion of the thoracic aorta (curve I), slightly decreased by occlusion of the brachiocephalic artery (curve II), moderately decreased by occlusion of the s.v.c. (curve III) and greatly decreased by occlusion of the i.v.c. (curve IV).

Fig. 4 shows that in the pump preparation comparable occlusions produced comparable changes in the systemic flow. The great vessels were occluded at the positions marked by *A*, *B*, *C* and *D* in Fig. 1.

A tracing of the events recorded in the pump preparation experiments during occlusion of the thoracic aorta is shown in Fig. 5. The occlusion

was made at *X*. The venous pressure rose sharply; this is shown in the top tracing. The pump was adjusted to bring the venous pressure back to its original level. This resulted in an increase in the arterial blood-pressure and in the systemic flow. The small jerks in the blood-pressure tracing are due to the reversal of the blood stream through the stromuhr.

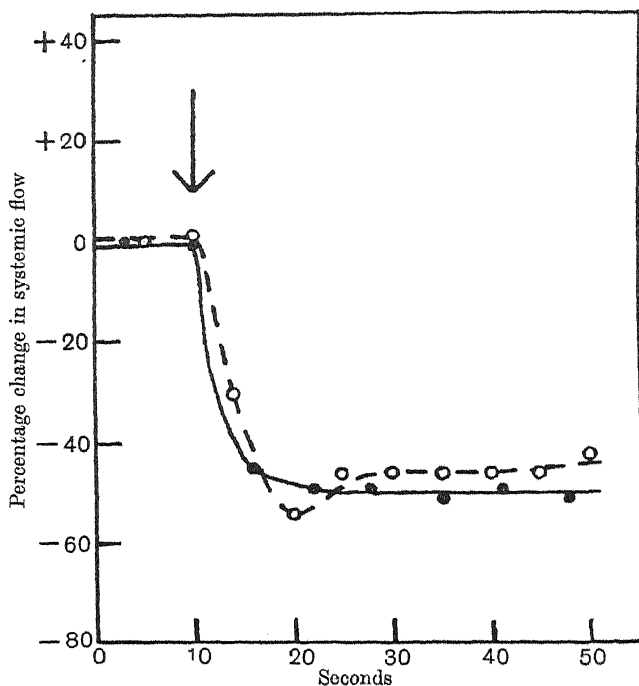


Fig. 6. Variations in the systemic flow in the animal and in the pump preparation after simultaneous occlusion of the thoracic aorta and the I.V.C. Continuous line: Animal. Initial flow, 410 c.c. per min. Broken line: Pump preparation. Initial flow, 590 c.c. per min. The occlusions were made at the time indicated by the arrow.

(3) *Simultaneous occlusion of the thoracic aorta and I.V.C.*

This procedure caused a considerable decrease in the systemic flow in the animal and in the pump preparation as shown in Fig. 6.

III. DISCUSSION.

The comparable behaviour of the systemic flow in the animal and in the pump preparation leads to the conclusion that the principles governing the action of the heart and of the pump were the same. Hence the

principles governing the pumping action of the heart in the animal were those enunciated by Starling for the heart in the heart-lung preparation.

IV. DESCRIPTION OF OPERATION FOR REPLACING THE HEART BY THE PUMP.

Blood was first obtained from two dogs, each weighing as much as possible. Two litres were sufficient.

Two other dogs, each weighing approximately 10 kg., were used in each preparation. Morphia was given. Equal parts chloroform and ether were used as anæsthetic.

Operation on the first animal. The first animal was used to prepare a heart-lung preparation. This preparation is described by Knowlton and Starling [1912]. The usual form of apparatus was used and was slightly elaborated as follows. The tube *E*, Fig. 7, opened into the tube leading from the heart-lung venous reservoir to the heart of the heart-lung preparation. At this stage of the operation it was clamped at *E*. The tube *P*, Fig. 7, opened into the tube leading from the finger-stall resistance to the venous reservoir of the heart-lung preparation. At this stage of the operation it was clamped at *P*.

Operation on the second animal. The animal was anæsthetized. Artificial respiration was established, using a Palmer's Ideal pump. Anæsthetic was administered through the pump as required. The thorax was opened along the mid-line. The internal mammary arteries, mediastinal vessels and thymus were cut between ligatures. The thoracic wall was divided on both sides between the fourth and fifth ribs. The ribs were drawn widely apart and secured. Two ligatures were placed round the i.v.c. The azygos vein was tied. Two ligatures were placed round the s.v.c. Two ligatures were placed round the brachiocephalic artery. The subclavian artery was tied between the aorta and the vertebral artery. A ligature was placed round the arch of the aorta below the subclavian artery. A ligature was placed round the aorta $1\frac{1}{2}$ in. below the ligature on the arch of the aorta. A ligature was placed round all the intercostal vessels arising from the aorta between the two ligatures round the aorta. A cannula leading to a burette was placed in the left auricular appendix. 0.25 g. of heparine in approximately 10 c.c. warm saline was injected through the burette. The arrangement of tubes and cannulæ attached to the pump and stromuhr is shown in Fig. 7. Artery forceps were placed initially at *B*, *C*, *F*, *G*, *P* and *E*. The whole apparatus was filled with blood at 40° C. This was added through the burette (Fig. 7). To assist this the pump was started at appropriate intervals, and air expelled by

loosening the artery forceps. The spiral was placed in a gas-heated water bath. The apparatus was attached to the animal as follows.

The upper ligature on the i.v.c. was tied. The cannula *H* was inserted peripherally and tied in by the lower ligature. The artery forceps at *C* was removed. The lower ligature on the s.v.c. was tied. The cannula *J*

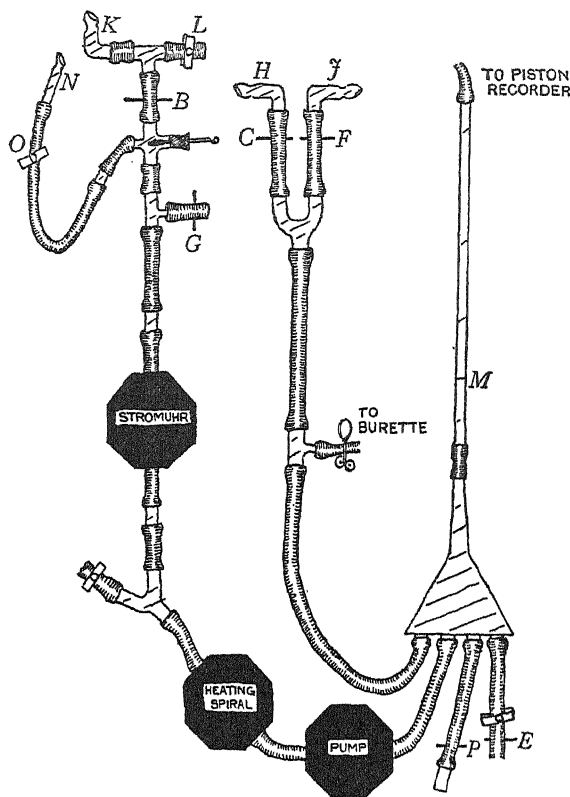


Fig. 7. Apparatus used in the experiments in which the dog's heart was replaced by a pump. Detailed explanation in text.

was inserted peripherally and tied in by the upper ligature. The artery forceps at *F* was removed. The ligature on the arch of the aorta was tied. A bull-dog clip was placed on the thoracic aorta below the lowest ligature and tightened. The cannula *K* was inserted peripherally, above the bull-dog and was tied in by the lower ligature. The artery forceps at *B* was removed. The pump was turned over a few times and air in the aortic cannula was driven out through the tube *L*. The clamp at *L* was then

tightened, and the bull-dog on the thoracic aorta removed. The arterial blood-pressure manometer was attached at *G*. The pump was started. Warm blood was added through the burette (Fig. 7) till the blood-pressure was 80 mm. of mercury and the blood was at the marked level in the tube *M* (Fig. 7). The lower ligature on the brachiocephalic artery was tied. The cannula *N* was inserted peripherally and tied in by the upper ligature. The screw clip at *O* was removed. The circulation of oxygenated blood from the heart-lung preparation through the reservoir was then established. The artery forceps at *P* and *E* were simultaneously removed, and were placed so as to prevent the blood entering or leaving the heart-lung venous reservoir. The flow through the heart-lung preparation was suitably adjusted by the screw clamp at *E*. The ligature round the intercostal vessels was tied. The preparation was now ready for the experiment.

The circulation in the animal was suspended for a few minutes after the ligatures on the venæ cavæ had been tied. During this period the brain was destroyed by asphyxia. By modifying the above technique this could have been avoided. Such a precaution was unnecessary, as the variations in the systemic flow studied were found alike in the animal with the vagi cut and the brain destroyed [Barcroft, 1931].

SUMMARY.

1. The systemic flow has been studied in the dog and in a preparation in which a pump replaced the dog's heart.

2. The output of the pump was adjusted so as to maintain the venous pressure approximately constant under all conditions. In this respect its action imitated the action of the heart in the heart-lung preparation under certain conditions described by Starling.

3. The behaviour of the systemic flow under a variety of conditions was found to be the same in the animal and in the pump preparation.

4. The conclusion drawn from these experiments is that, under the conditions studied, the animal's heart reacted to artificial changes in the peripheral circulation by adjusting its output so as to maintain the venous pressure approximately constant.

I am very grateful to Prof. Barcroft for much kind help in this research.

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THE DECEREBRATE RAT.

BY D. J. BELL¹, E. A. HORNE AND H. E. MAGEE.

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THIS research was begun by two of us (E. A. H. and H. E. M.) with the object of obtaining a decerebrate-rat preparation suitable for the study of intestinal absorption and with an approximately normal blood sugar level. In such a preparation solutions of the substances under investigation could be injected directly into the small intestine and the complicating factor of the rate of emptying of the stomach thereby avoided. Evidence has indeed been brought forward by Macleod, Magee and Purves [1930] and by McSwiney and Pyrah [1932] suggesting that the rate of emptying of the stomach depends in some measure on the solute concentration of its contents.

Donhoffer and Macleod [1932] have shown that in the rabbit, hyperglycæmia follows decerebration through the pons but not through any other region of the brain. Similar results have been obtained in the cat by Peterson [1932]. It was therefore necessary, at the outset of this investigation, to ascertain whether similar results were obtainable in the rat. It, however, soon became evident that decerebration at any level of the brain failed to elicit hyperglycæmia. The research was then continued by the third author (D. J. B.) to find out whether, by varying the experimental and dietary conditions, hyperglycæmia could be obtained in rats by brain section. The paper is, therefore, divided into two parts: Part I by E. A. H. and H. E. M. and Part II by D. J. B.

PART I. DECEREBRATION OF THE RAT UNDER LOCAL ANÆSTHESIA
AND THE USE OF THE PREPARATION FOR ABSORPTION STUDIES.*Method.*

It was found, from observations made on dead animals, that the pons or mid-brain could be readily sectioned through a trephine hole in the skull, made about 2 mm. behind the posterior end of the sagittal suture.

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Usually 0.2 c.c. of 5 p.c. cocaine was carefully injected deep under the skin, along a line extending from just in front of the occipital protuberance to a line joining the ears. About 10 min. later the skin was incised along the line of injection for about 2 cm. and the skull bared. The rat was then released from the firm grip in which it was held, in order to allow the venous congestion in the head to subside. After a few minutes the animal was gripped as before and the lower jaw placed on the edge of the table. A hole was then rapidly bored in the skull by rotating rapidly to and fro a very sharp blood-sampling stilette. The stilette was broad enough to make a hole about 4 mm. in diameter. A blunt dissector was then inserted into the opening and directed so as to sever the brain in the position desired. This portion of the operation generally required

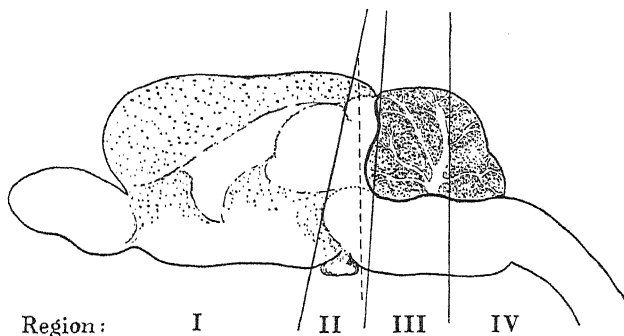


Fig. 1. Sagittal section of rat's brain.

somewhat less than 5 sec. The rat was immediately inverted and its head kept at a lower level than the rest of the body to prevent blood trickling down towards the medulla. Manual artificial respiration was often necessary for a few minutes at this stage. When spontaneous respirations were resumed the animal was placed on the operating table, the heated plate of which was inclined so that the rat's head was lower than the rest of its body.

The survival period of animals prepared in this way was variable. About 25 p.c. died within an hour after operation, the usual cause of death being, in so far as one could judge, compression of the medulla due to blood clots. As a rule the majority lived 4-5 hours and often preparations were killed after 6 hours when still in good condition, as judged by the degree of oxygenation of the blood. Care had to be exercised to prevent overheating or cooling, both of which adversely affected the viability of the preparation. The loss of blood rarely amounted to more

than 0.5 c.c. and it occurred mainly during the making of the trephine hole. Spontaneous movements were generally absent unless the section was made anterior to the corpora quadrigemina. At the conclusion of each experiment the animal was decapitated and the blood collected for sugar estimation (Hagedorn-Jenson method). The skull and brain were then split sagittally and the position of the section located.

In describing the site of brain section the terms employed by Donhoffer and Macleod are used, *i.e.* region I = anterior to the corpora quadrigemina; region II = the mid-brain; region III = the pons, and region IV = the medulla (see Fig. 1). Very few decerebrations were made in region I because of the great excitability of such preparations.

Results.

Effect of decerebration on the blood sugar of rats fasted 24 hours.

	Normal	Decerebrated	
		Region II	Region III
No. of rats	12	5	10
Average blood sugar mg./100 c.c.	82	90	95
Variations, mg./100 c.c. ...	72-94	78-102	75-109
Killed at hours after decerebration	Not decerebrated	2-5.25	2-4.75

The above results show that decerebration in regions II and III had no appreciable effect on the blood-sugar level. In two other animals decerebrated in region III the blood sugar rose to 129 and 140 mg., the respective animals having been decerebrated 2 and 4.75 hours previously. Except in these two animals there was no correlation of any kind between time after decerebration and blood-sugar level. Donhoffer and Macleod found that hyperglycæmia was most marked between 2 and 4 hours after pontine injury in the rabbit. It is therefore clear, since the rats decerebrated in region III were killed at varying times between 2 and 4.75 hours afterwards, that pontine section either does not cause hyperglycæmia in rats fasted 24 hours or that hyperglycæmia occurs earlier and is of shorter duration than in the rabbit. To investigate this possibility, six similarly fasted animals were killed within 30 min. after pontine decerebration, but the average blood sugar was only 102 mg. with variations between 92 and 108 mg.

Effect of decerebration on fed rats.

It appeared possible that the failure to elicit hyperglycæmia was due to inadequate glycogen stores in the rats. Decerebrations were accordingly performed on animals which had had access to their usual stock

diet until 30 min. before operation. This diet consisted of cereals, fish, meat and bone meals, dried milk, yeast, cod-liver oil, NaCl and green stuff *ad lib.* daily. The vitamin content is adequate and, excluding the green stuff, the ratio, protein : fat : carbohydrate is approximately 1 : 0.13 : 1.8 and the ratio, CaO : P₂O₅ : K₂O is 2.7 : 3.5 : 1.

	Normal	Decerebrated	
		Region II	Region III
No. of rats	6	5	7
Blood sugar	93	104	94
Variations	—	80–121	76–102
Killed at hours after decerebration	Not decerebrated	3.5–4.75	3.3–4.25

These results show that decerebration had no effect on the blood sugar of fed rats. The blood-sugar values for the normal animals appeared to be too low for the absorptive state and suggested that the stock diet was not rich enough in carbohydrate for adequate stores of glycogen to be laid down. Another group of rats was therefore fed for a day on a strong solution of cane sugar in milk *plus* a smaller than usual helping of their stock ration. They had had access to this diet until about 30 min. before decerebration. The mean blood sugar of five normal (non-decerebrated) rats was 128 mg. (variations 118–132), that of the five decerebrated rats (region III only) was 131 mg. (variations 118–142). The latter were killed 1.5 hours after operation. This result is somewhat puzzling in view of the fact, as shown in Part II, Tables VI and VII, that pontine puncture caused hyperglycemia in rats previously fed several days on maize or potatoes.

We concluded from the foregoing results that pontine decerebration was without effect on the blood sugar of rats whether fasted or fed. In so far as we (E. A. H. and H. E. M.) were concerned, therefore, the main objective of the research had been attained, and it only remained for us to test whether the glucose absorption coefficient in such preparations was reasonably constant.

Absorption coefficient for glucose introduced directly into the intestine of decerebrate rats.

The abdomen of rats fasted for 24 hours and decerebrated, as above, 30–60 min. previously was opened along the linea alba, a glass cannula inserted into the duodenum *via* a slit in the pylorus and secured by a double ligature of woollen yarn tied round the sphincter. A piece of rubber tubing, carrying a clip, was fastened on to the free end of the

cannula, to facilitate insertion of fluid into the intestine. The end of the ileum was tied to prevent the test solutions passing into the large intestine, in which, as is known, little or no absorption takes place. The abdomen was sewn, leaving the rubber tubing projecting from the anterior end of the wound.

Blank experiments were carried out on seven rats to find out whether the blood sugar was affected by the operation. The animals were killed 30 min. after completion of the abdominal operation. The mean blood sugar was 128 mg. with variations of 116–152. This result shows that the abdominal operation raised the blood sugar but not to a marked extent.

In the absorption tests 3 c.c. of 0.75 *M* glucose at 40° were injected into the duodenum. An hour later the animals were killed and the absorption coefficient determined in the usual way. Random examples of results and mean values are given below.

Absorption coefficient (A.C.) and blood sugar (B.S.) of rats fasted 24 hours, decerebrated; 60 min. later 3 c.c. 0.75 *M* glucose placed in duodenum and killed 60 min. after feeding.

Site of decerebration ...	Region II		Region III		Region IV	
	A.C.	B.S.	A.C.	B.S.	A.C.	B.S.
	0.106	250	0.160	188	0.102	221
	0.121	141	0.089	246	0.097	262
	0.148	203	0.225	380	0.141	279
	0.159	350	0.128	227	0.139	258
Average ...	0.136	250	0.146	266	0.120	255
No. of results for average	10		7		4	
Variations ...	0.106–0.190	141–361	0.089–0.225	152–382	0.097–0.141	221–279

The differences between the A.C.'s for animals decerebrated in regions II and III are distinctly higher than for those done in region IV. The reason probably is that post-pontine section was not generally well borne. The values for region II were the most regular and they are not significantly different from those for region III. The mean A.C. for region II, 0.136, is, however, significantly lower (22 p.c.) than that found by Magee and Sen [1932] in intact animals. There are several causes to which this difference could be ascribed, but discussion of them is premature.

There was no clear relationship between the A.C. and the blood-sugar level. The values for the latter are very irregular and they suggest that some factor incidental to the operative measures employed was causing a disturbance of carbohydrate metabolism.

PART II. HYPERGLYCÆMIA FROM BRAIN SECTIONS IN THE RAT.

In view of the negative character of the results of Part I, it was decided to investigate the ultimate effect on the blood-sugar level, of decerebration performed under general anæsthesia, in order to avoid the possible effects of shock to the nervous system caused by transection or piqûre in a condition of superficial anæsthesia.

A. *Blood-sugar level of intact animals under amytal anæsthesia.*

In this group the rats were fed solely on the stock diet until within either 3 or 24 hours of the commencement of the experiment, the former being designated "fed" and the latter "fasted" animals. Anæsthesia was induced by intraperitoneal injection of 2 p.c. amytal solution, dose 10 mg./100 g. body weight. After the first 90 min. half-hourly injections of one-fifth of the initial dose were given, to maintain anæsthesia. Blood samples of 0.10 c.c. were withdrawn from the carotid artery through a cannula so designed that only minute amounts of blood were lost. The results of these observations are collected in Table I, they show a difference of about 20–30 mg. p.c. sugar between fed and fasted animals, and in both groups a remarkably steady sugar level at varying periods after the commencement of anæsthesia.

TABLE I.
Blood sugar (mg./100 c.c.)

Time after giving amytal min.				
	Fed animals (mean of 3 expts.) (Variation)		Fasted animals (mean of 3 expts.) (Variation)	
15	134	132–137	—	—
30	136	133–137	112	87–121
45	136	130–140	—	—
60	140	135–145	110	80–120
90	146	145–147	—	—
120	—	—	97	79–110
135	141	140–142	—	—
150	141	140–142	107	80–121
180	143	Nil	—	—
195*	143	142–144	—	—
240	143	Nil	119	99–122

B. *Effect on the blood-sugar level of decerebration in regions II and III.*

Fed and fasted animals were used, anæsthetized by intraperitoneal injection of 7 mg./100 g. body weight amytal as 2 p.c. solution. This produced deep anæsthesia for about 45 min. during which the necessary operations were performed. Cannulæ were inserted into the trachea and

one carotid artery, the intact carotid clipped and the brain freely exposed by trephining anterior to the occipital protuberance. A cut was made in the dura mater avoiding the superior longitudinal sinus, and, with an almost straight aneurysm needle, complete transection of the brain stem was made at the desired level. Hæmorrhage was usually copious unless the vertebral arteries were compressed, when interruption of respiratory movements often followed. Restoration of the blood supply to the brain, by releasing both the vertebral and carotid arteries, usually resulted in a further, and frequently fatal, loss of blood. Mortality over the whole series of experiments was about 30 p.c. Table II gives observations made on preparations, decerebrated in region II, showing spontaneous respiration and good condition 150 min. after the operation.

TABLE II.

Time after decere- bration min.	Blood sugar (mg./100 c.c.)			
	Fed animals (mean of 4 exps.)		Fasted animals (mean of 8 exps.)	
		(Variations)		(Variations)
30	151	141-160	100	82-110
45	—	—	99	81-121
60	148	137-161	105	82-123
90	145	140-165	110	80-110
120	137	130-151	95	81-109
150	140	131-153	98	79-108

The above values are exactly comparable with those of the corresponding intact animals (Table I). Seven animals survived decerebration in region III, but hyperglycæmia developed in only one of them.

The unsatisfactory nature of the foregoing experiments led to the adoption of an entirely different technique in which ether was used to induce temporary anæsthesia, and the decerebration effected by Schmidt's [1923] method. The author wishes to thank Dr J. M. Peterson for suggesting the use of this method and for assisting him in carrying out the preliminary experiments in its application to the rat.

C. *Decerebration by Schmidt's method during ether anæsthesia.*

Preliminary experiments showed that, in preparations decerebrated in region II, under ether anæsthesia, the post-operative hyperglycæmia rapidly disappeared. Schmidt's method was applied to the rat in the following manner: The animal was anæsthetized under a bell-jar and a tracheal cannula introduced. Two discs were defined on the exposed parietal bones of the skull, by means of a small trephine and the discs carefully picked out exposing the dura mater intact. A thread was

passed through one hole, through the dura across the upper surface of the brain but below the superior longitudinal sinus, and out at the opposite hole. Using a strong, slightly curved needle, the two ends of the thread were passed through the holes and downwards, towards the line of the angles of the jaw, till the needle-point, after penetrating the roof of the mouth, emerged beneath the lower jaw, between the mandibles. The two ends of the thread were drawn tight and tied. By this procedure the brain was found to be completely transected in region II as indicated by the dotted line in Fig. 1, with the loss of only very small amounts of blood. The success of the method may be gauged from the following considerations, in addition to the absence of hæmorrhage.

I. Out of 60 experiments only three deaths occurred as a result of the operative technique¹.

II. In the remaining 57 experiments spontaneous respiration failed in only two instances.

(1) *Experiments on animals fed on the stock diet.*

(a) *The effect of region II decerebration followed by pontine (region III) decerebration.* After a suitable post-operative recovery period had elapsed, blood samples were withdrawn from the femoral artery in groups of fed and fasted animals. No marked degree of hyperglycæmia was observed following decerebration in region II except in one or two preparations. In these animals the stomachs were distended by food so that the hyperglycæmia was probably alimentary. A series of experiments was performed where the animal was decerebrated in region II, the blood sugar determined after a time and then a lesion produced in the pons by means of an aneurysm needle. Typical results are shown in Table III.

Respiration, heart beat and blood-pressure were well maintained subsequent to decerebration in region II; lesions in the pons, however, were frequently followed by irregularity, or complete cessation of respiratory movements, and by marked acceleration of the heart rate. It is evident that decerebration either in region II or region III did not evoke hyperglycæmia.

(b) *The effect of removal of the cerebellum, followed by pontine puncture in animals decerebrated in region II.* This was next investigated, both in fed and fasted animals. After a recovery period following decerebration, as described above, the blood sugar was estimated, the greater

¹ Subsequent experiments on animals which had been rigorously fasted, 36-24 hours, showed that mortality was higher than found under the above conditions.

TABLE III.

Note regarding tabulation of results in this and in subsequent tables: *Time* is reckoned in minutes after completion of decerebration in region II and is indicated by the small index figures above the blood-sugar values—*e.g.* 100⁶⁰ signifies 100 mg./100 c.c. at 60 min. after decerebration. (*P*) signifies pontine decerebration or puncture, the index figures referring to the time after the first decerebration.

		Blood sugar mg./100 c.c.					
Exp.		Fed animals.					
1	147 ³⁰	(P ³⁵)	147 ⁵⁰				
5*	185 ⁴⁰	235 ⁷⁰	(P ⁷¹)	210 ⁹⁰			
7	115 ¹⁵	125 ²⁵	135 ⁵⁵	(P ³⁵)	108 ⁸⁵	117 ¹²⁰	
		Fasted animals.					
3	137 ²⁰	118 ⁴⁰	(P ⁴⁵)	105 ⁶⁵			
6	110 ³⁰	90 ⁶⁰	(P ⁶⁵)	85 ⁸⁰			
4	103 ³⁰	(P ³⁵)	(97 ⁴⁵)	75 ⁵⁵			

* In this animal the stomach was found to be distended with food at the conclusion of the experiment.

portion of the occipital and parietal bones cut off and the cerebellum carefully removed. This operation was usually accompanied by violent movement and care had to be taken that the pons was not accidentally injured. Voluntary respiration was usually impaired and the heart rate accelerated; if necessary the preparations were artificially respired. The operation was seldom accompanied by more than a small loss of blood.

It was found that in no instance was the blood-sugar level raised by this procedure. Further experiments were carried out where the exposed pons was punctured, on one side or the other, or completely transected. This procedure also failed to produce hyperglycæmia. The results of this series of experiments are detailed in Table IV.

TABLE IV. *CR* signifies "cerebellum removed."

		Blood sugar (mg./100 c.c.) at minutes after decerebration.					
Exp.		Fasted animals.					
9	110 ³⁰	102 ⁴⁵	<i>CR</i> ⁴⁵	111 ⁵⁵	62 ⁷⁰		
27	110 ³⁰	<i>CR</i> ³⁵	108 ⁵⁰	100 ⁷⁰			
15	80 ⁴⁵	<i>CR</i> (P) ⁵⁰	67 ⁸⁰	45 ⁹⁵			
		Fed animals.					
12	180 ³⁰	<i>CR</i> ³⁵	150 ⁵⁰	155 ⁶⁰			
13*	213 ³⁰	<i>CR</i> ⁴⁰	187 ⁶⁰	166 ⁷⁵			
14	188 ⁴⁰	<i>CR</i> ⁴²	144 ⁶⁰	81 ⁷⁰	(P) ⁷⁵	45 ⁹⁰	53 ¹⁰⁰
26	173 ⁷⁰	<i>CR</i> ⁷⁵	192 ¹⁰⁵	(P) ¹¹⁰	138 ¹³⁵	158 ¹⁵⁰	
24	129 ⁴⁰	<i>CR</i> (P) ⁵⁰	117 ⁷⁰				
25	147 ⁴⁵	<i>CR</i> (P) ⁵⁰	141 ⁷⁰				

* Stomach distended with food, at conclusion of experiment.

(c) *The change in liver glycogen resulting from the above operative treatment.* The liver glycogen of a number of unoperated animals, receiving preliminary feeding under conditions exactly similar to those employed in the foregoing experiments, was determined by the Pflüger method, the animals being killed by decapitation. The liver glycogen of the fed decerebrate preparations of Table IV was also determined at the close of the experiments, and comparison of the two showed that the operative procedure employed brought about a glycogen decrease (see Table V).

TABLE V. Liver glycogen (g./100 g.) in fed animals.

Unoperated controls (mean of 5)	0.80 variations 1.31-0.50
Operated animals after experiment (mean of 6)			0.07 variations 0.08-0.06

Consideration of similar work on the rabbit at present in progress in this laboratory, coupled with the unexpectedly low initial glycogen content of the unoperated animals led the author to repeat the pontine-puncture experiments with animals fed on a diet richer in carbohydrate so as to raise the glycogen content of the liver. To this end, the stock diet was augmented by the addition either of (a) two-thirds of its bulk of crushed maize, or (b) an equal bulk of boiled potatoes. Animals were divided into two groups and fed for several days, each on one of the augmented diets. Liver glycogen values for the unoperated animals were then determined as before.

TABLE VI. Liver glycogen (g./100 g.) in animals fed on carbohydrate augmented diets (a) and (b).

Diet (a)	Stock plus maize (mean of 5)	2.94
Diet (b)	Stock plus potatoes (mean of 4)	3.50

(2) *Experiments on animals fed on the carbohydrate-augmented diets.*

Food was removed from the animals 5 hours before the experiments which were conducted exactly as described in section C (a) and (b) above. As will be seen from consideration of Tables VII and VIII decerebration in region II was followed by no marked hyperglycæmia, nor did removal of the cerebellum cause any rise in the previous blood-sugar level. Pontine lesions, however, in 12 cases out of 14 brought about a marked and sustained rise.

TABLE VII. Diet augmented with maize.

Exp.	Blood sugar (mg./100 c.c.) at times after decerebration.						
30	99 ⁴⁵	CR ⁵⁰	132 ⁷⁰	129 ¹⁰⁰			
31	132 ⁴⁵	CR ⁵⁵	117 ⁸⁵				
33	180 ⁴⁵	CR ⁵⁰	174 ⁷⁵	136 ⁹⁰	90 ¹²⁰		
16	185 ⁵⁵	CR(P) ⁶⁰	300 ⁹⁰				
17	181 ³⁰	CR(P) ⁵⁰	312 ⁷⁰				
18	168 ⁶⁰	CR(P) ⁷⁰	203 ⁸⁵				
19	87 ³⁰	CR(P) ⁶⁵	265 ⁹⁰	258 ¹²⁰			
20	174 ⁶⁰	CR(P) ⁶⁵	210 ⁸⁰				
21	131 ⁶⁰	CR(P) ⁶⁵	157 ⁷⁵	231 ⁹⁰	241 ¹⁰⁵	216 ¹²⁵	231 ¹⁴⁵
22	204 ⁴⁵	CR(P) ⁶⁰	285 ⁷⁰	141 ⁸⁵	108 ¹¹⁵		
23	132 ⁴⁵	CR(P) ⁵⁰	204 ⁶⁰				
28	147 ⁴⁵	CR(P) ⁵⁰	207 ⁵⁰				
29	137 ³⁵	CR(P) ⁴⁰	114 ⁵⁵	207 ⁸⁰	275 ⁹⁵		
32	107 ³⁵	CR(P) ⁴⁰	180 ⁵⁵	276 ⁸⁵			

Liver glycogen, determined at conclusion of experiment.

Cerebellum removed ... 0.73 p.c. (mean of 2 exps.).

Cerebellum removed and pons punctured 0.08 p.c. (mean of 7 exps.).

Fall in liver glycogen of fed animals after pontine decerebration, from 2.94 to 0.08 p.c.

TABLE VIII. Diet augmented by potatoes. Cerebellum left intact before pontine puncture.

Blood sugar (mg./100 c.c.) at times (min.) after decerebration.				
Exp.	Animals fasted 24 hours.			
34	84 ⁴⁵	(P) ⁶⁰	80 ⁹⁰	
35	105 ⁶⁰	(P) ⁶⁵	70 ⁹⁵	
36	105 ³⁵	(P) ⁴⁰	80 ⁶⁰	80 ⁹⁰
"Fed" animals (food removed 5 hours).				
37	170 ⁴⁵	(P) ⁵⁰	243 ⁷⁰	250 ⁹⁵ 250 ¹¹⁵
38	190 ⁴⁰	(P) ⁴⁵	288 ⁶⁰	266 ⁸⁰ 330 ¹¹⁵
40	108 ⁶⁵	(P) ⁷⁰	132 ¹⁰⁰	
41	168 ³⁰	(P) ³⁵	184 ⁵⁵	225 ⁸⁰

Liver glycogen, determined at conclusion of experiment.

Fasted animals ... 0.05 p.c. (mean of 2 exps.).

Fed animals ... 1.31 p.c. (mean of 3 exps., Nos. 37, 38, 41).

Fall in liver glycogen in fed animals after pontine decerebration, from 3.50 to 1.31 p.c.

DISCUSSION.

It is clear from both groups of experiments that decerebration through the pons, whether performed under local or general anæsthesia, failed to produce hyperglycæmia in rats fed on a well-balanced stock ration. When the diet contained a large excess of carbohydrate (maize or potato), lesions in this region of the brain caused hyperglycæmia as in rabbits. It is, however, curious that hyperglycæmia did not result from pontine decerebration in the rats previously fed on cane sugar. The probable cause of the failure was that the rats did not appear to relish the sugary diet; and it may be also that the period of feeding, 24 hours, was too

short. It is, however, clear that the liver glycogen content was the determining factor, for the mean values in unoperated control rats fed on the different diets were: stock 0.8 p.c., maize 2.9 p.c. and potato 3.5 p.c. It is impossible, as yet, to explain why the occurrence of hyperglycæmia after pontine injury should depend in the rat, but not in the rabbit, on previous feeding with carbohydrate-rich diets. It does, however, appear to be established that the pons is the only region of the brain in which puncture or section is followed by hyperglycæmia in rabbits, cats and rats.

SUMMARY.

Decerebrate-rat preparations, which can be used for studies of carbohydrate absorption and metabolism, have been obtained by section of the brain stem carried out under local or general anæsthesia. At whatever level it is performed decerebration is not followed by hyperglycæmia in rats fed on a well-balanced diet. In rats fed previously on diets consisting largely of maize or potatoes, damage to the brain caused hyperglycæmia, but only when the pons was involved. The liver glycogen in unoperated control rats was about four times as great in those fed on maize or potato supplements as in those fed on the balanced diet only. Rich stores of liver glycogen would therefore appear to be necessary for the causation of pontine hyperglycæmia in the rat.

The absorption coefficient for glucose, in 0.75 *M* concentration placed directly into the intestine of decerebrate rats, is lower than when the sugar is given by stomach tube to normal intact rats.

We wish to record our indebtedness to Prof. J. J. R. Macleod for his constant interest and helpful advice, and to Miss M. L. Long for carrying out the glycogen determinations of Part II.

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THE RESPONSES TO STIMULATION OF THE
CAUDAL END OF THE LARGE BOWEL
IN THE CAT.

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OUR knowledge of the phenomena of defæcation is derived mainly from work on man. In few other fields of Physiology has clinical observation played so large a part. Unfortunately, the study of clinical material is at the mercy of the capriciousness of disease processes and of accident. Thus systematic investigation in man of the factors controlling the behaviour of the caudal end of the large bowel is well-nigh impossible. On the other hand, observations on lower animals are scanty, due mainly to the absence of a satisfactory mode of stimulation capable of eliciting defæcation responses.

The terminology used here is, in the main, that recommended by Langley and Anderson [1896]. The origin and course of the nervous outflows to the large bowel in the cat are illustrated in a previous paper [Garry, 1933]. In the cat, however, it is not justifiable, as Elliott and Barclay-Smith [1904] point out, to divide the colon into regions corresponding to those of human anatomy. Nevertheless, it is convenient to refer to the cranial and caudal regions of the large bowel and to regard the anal canal as that part encircled by the anal sphincters. The name external anal sphincter should be applied to the entire striped muscle innervated by the pudendal nerves, and the name internal and sphincter confined to the circular aggregation of smooth muscle fibres at the anus. The external anal sphincter overlaps the internal anal sphincter and surrounds the anal glands. The levator ani in man is the homologue of the pelvo-caudal muscles which flex and abduct the tail in the cat.

PREVIOUS WORK.

The influence of the spinal cord on defæcation.

Gowers [1877] experimented on three men, two of whom suffered from a complete transverse lesion of the spinal cord. In these two patients irritation of the rectal mucous membrane or distension of the rectum by a puff of air caused dilatation of the anal canal. Head and Riddoch [1917] and Riddoch [1917] found that in spinal man, after recovery from the long period of spinal shock, distension of the rectum led to defæcation.

Goltz and Freusberg [1874] showed that the large bowel in spinal dogs is able to expel foreign bodies. Sherrington [1900] states that defæcation occurs normally in spinal mammals and that stimulation of the rectal mucous membrane cranial to the anal canal leads to relaxation of the anal sphincters. Apparently, then, defæcation is essentially unaffected by transection of the spinal cord cranial to the lumbar region.

On the other hand, after destruction of or injury to the lumbo-sacral region of the spinal cord, defæcation is affected, although the large bowel is still able to expel its contents. The third patient of Gowers suffered from injury to the dorsal roots of all the sacral nerves and to both roots of the caudal sacral nerves. The circumanal region was insensitive, there was incontinence of fæces and the external anal sphincter was paralysed. In this man, as in the other two men, irritation of the rectum caused dilatation of the anal canal. Goltz and Ewald [1896] claimed that defæcation ultimately became normal in their "cordless" dogs. Bayliss and Starling [1900] with difficulty obtained "descending inhibition" and "ascending excitation" in the decentralized large bowel of dogs. In rabbits similar responses were more easily obtained. It is doubtful, however, if such responses can be regarded as true defæcation responses since Bayliss and Starling deliberately avoided the anal canal because its control is "more intimately connected with the central nervous system." Elliott and Barclay-Smith [1904] observed that excretion of fæces was quite satisfactory, and that irritation of the anal mucous membrane caused extrusion of fæcal pellets in rats, even after destruction of the lumbo-sacral spinal cord. Nevertheless, these authors concluded that decentralization does disturb the normal coordinated activity of the large bowel.

The peripheral nerves concerned with defæcation.

Head [1893], from a study of pain in visceral disease, came to the conclusion that the sensory inflow from the rectum in man passes by the dorsal roots of the second, third and fourth sacral nerves. Langley and Anderson [1895] found that cutting the sacral nerve roots abolished voluntary defæcation in lower animals. Although his primary interest was in the behaviour of the tail, Merzbacher [1902] noted that, after section of the dorsal roots of the sacral and tail nerves, the presence of a foreign body in the rectum led neither to expulsion of that body nor to adoption of a typical somatic defæcation posture.

Barrington [1915], on the other hand, found that cats, with the pelvic and pudendal nerves both cut, still had the desire to defæcate and that fæcal matter did not accumulate in the large bowel. In spite of this fact, Barrington believes that the main afferent pathway for the impulses leading to defæcation lies in the pudendal nerves.

The nature of the stimulus for defæcation.

It is generally accepted that defæcation is elicited by stimulation of the caudal end of the gut. Zimmermann [1909] and Hertz [1911] believe that distension of the rectum is the stimulus in man, and Cannon [1911] states that distension of the caudal end of the large bowel initiates defæcation in lower animals. Garry [1932] elicited defæcation by stimulation of the distal part of the large bowel in decerebrate cats.

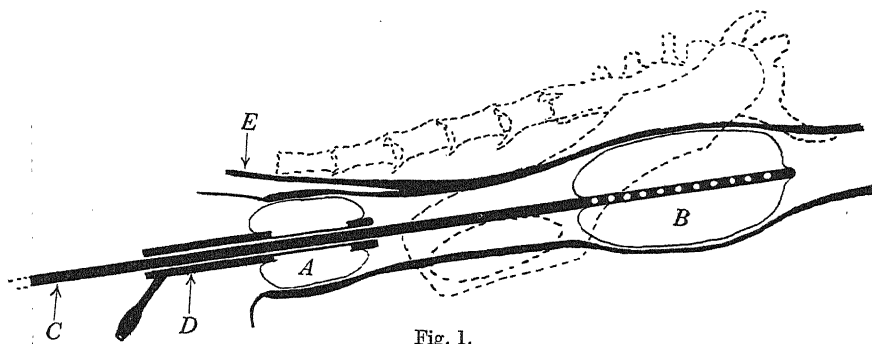
Lehmann [1913] found that stimulation of the central ends of various somatic nerves led to contraction of the cranial and to relaxation of the caudal part of the large bowel in dogs. The integrity of the pelvic nerves was necessary for such responses. Stimulation of the central ends of visceral nerves supplying the large bowel itself, however, did not cause this "defæcation" response. As a result Lehmann came to the somewhat surprising conclusion that the stimuli initiating the act of defæcation do not act on the large bowel. This conclusion is accepted by Ranson [1921].

METHODS.

The preparation of the cats and the operative technique have already been described [Garry, 1933]. The movements of the large bowel and of the anal canal were recorded by two tandem balloons inserted through the anus (Fig. 1). Recording was either by volume changes at a constant pressure in the neighbourhood of 21 cm. water pressure or by pressure changes at a constant volume. Simple distension of the balloons, both

in the large bowel and in the anal canal, rarely elicited responses. On the other hand rotation or a slight to-and-fro movement of the balloons, distended or undistended, almost invariably elicited marked response which had as its obvious purpose expulsion of the stimulating balloon. Such stimulation, although not recognised as normal according to currently accepted views, does enable one to elicit defæcation responses with ease and with certainty and thus to investigate the part played by the various extrinsic factors in defæcation.

The holder for the balloon in the large bowel moved freely within the holder for the balloon in the anal canal. It was thus possible to move one balloon without moving the other balloon. The holder of the



The recording balloons. *A*, the balloon in the anal canal; *B*, the balloon in the large bowel; *C*, holder for balloon in the large bowel; *D*, holder for balloon in anal canal; *E*, recto-coccygeus muscle.

stationary balloon was firmly grasped by a clamp attached to the operating table. In this way it was possible to stimulate, and, if the stimulating balloon were distended, to record the local response to the stimulation as well as the response at a distance.

The balloon within the large bowel was 4 cm. long and the balloon within the anal canal 2 cm. long. Usually 4 to 6 cm. separated the cranial end of the balloon in the anal canal from the caudal end of the balloon in the colon.

In several cats the balloon for the large bowel was introduced on a short metal holder through a colostomy in the left flank. The colostomy was close to the ileocolic sphincter. In such cases rubber tubing connected the balloon to the recording system. The responses obtained did not differ from those elicited by the simpler and less objectionable technique, although, due to injury to the peritoneum, it was usually impossible in

cats with a colostomy to produce any response before division of the lumbar outflow.

When necessary curare was given by slow intravenous injection of a 0.05 p.c. suspension.

RESULTS.

(1) *The response of the large bowel to stimulation of the large bowel.*

Movement of the balloon within the large bowel causes contraction of the large bowel. Unless the stimulus is prolonged or vigorous, there is

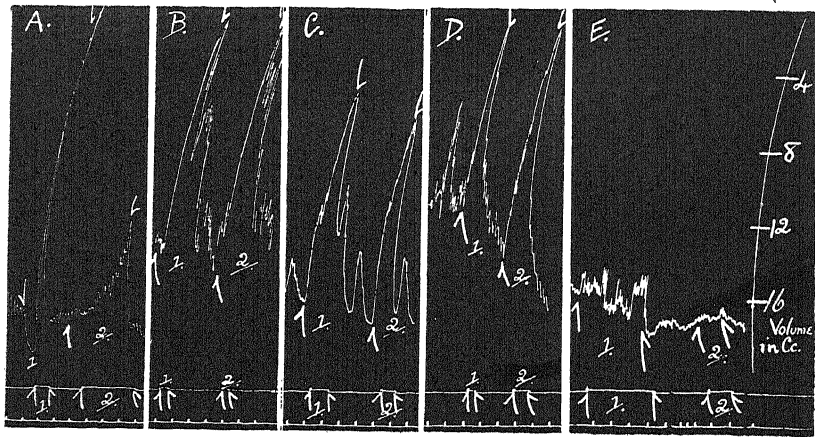


Fig. 2.

Cat: decerebrate; volume record from large bowel at constant pressure of 21 cm. H_2O .
Time records in minutes.

- A. (1) Collapsed caudal balloon moved to and fro in anal canal. (2) Distended balloon rotated within large bowel.
- B. Pudendal nerves and lumbar outflow cut. (1) Collapsed caudal balloon moved to and fro in anal canal. (2) Distended balloon rotated within large bowel.
- C. Lumbo-sacral cord isolated. (1) Collapsed caudal balloon moved to and fro in anal canal. (2) Distended balloon rotated within large bowel.
- D. Full curarization induced. (1) Collapsed caudal balloon moved to and fro in anal canal. (2) Distended balloon rotated within large bowel.
- E. Spinal anaesthesia induced. (1) Distended balloon rotated within large bowel. (2) Collapsed caudal balloon moved to and fro in anal canal.

rarely any sign of participation of the somatic muscles in the response. Such contraction of the large bowel may be elicited in anaesthetized cats (Fig. 4 B), in decerebrate and in decapitate cats (Figs. 2 A(2)), and in cats with isolated lumbo-sacral cord (Fig. 2 C(2)). Full curarization does not affect the response (Fig. 2 D(2)).

The response is more easily elicited and is of greater magnitude after division of the lumbar outflow to the large bowel (Fig. 2 A(2), B(2)).

After section of the pelvic nerves (Fig. 4 D) and after induction of spinal anæsthesia (Fig. 2 E(1)) the large bowel usually fails to respond to stimulation. When the bowel does respond, the contraction is fugitive and difficult to elicit. Abolition of the response during spinal anæsthesia and reappearance of the response after disappearance of the spinal anæsthesia is shown in Fig. 5 B.

Application of 5 p.c. cocaine hydrochloride to the mucous membrane of the large bowel causes contraction of the gut for some time and abolishes all response to stimulation.

(2) *The response of the anal canal to stimulation of the large bowel.*

Movement of the balloon within the large bowel causes relaxation of the anal canal. This occurs in anæsthetized cats (Fig. 4 B), in decerebrate cats (Fig. 3 A(1)), in decapitate cats (Fig. 3 C) and in cats after transection of the cord in the lower thoracic region (Fig. 3 B(1)). Dilatation of the anal canal occurs after division of the pudendal nerves (Fig. 3 A, B). Section of the lumbar outflow facilitates the response (Fig. 3 C). Full curarization does not affect the response (Fig. 3 B(3)).

After induction of spinal anæsthesia (Fig. 3 B(7)) and after section of the pelvic nerves (Fig. 3 A(3)) stimulation of the large bowel is without effect on the anal canal. Application of 5 p.c. cocaine hydrochloride to the mucous membrane of the large bowel abolishes this response.

Two cats, both decerebrate, gave responses out of keeping with the above description. In both apparently complete section of all outflows to the large bowel, combined with spinal anæsthesia, did not abolish dilatation of the anal canal on stimulation of the large bowel cranial to the anal canal.

(3) *The response of the anal canal to stimulation of the anal canal.*

A to-and-fro or rotary movement of the balloon within the anal canal causes relaxation of the anal canal. Such stimulation of the anal canal in uncurarized cats, in marked contrast to stimulation of the large bowel, is usually accompanied by active participation of the somatic muscles in the response. If anything, a to-and-fro movement is more potent than a rotary movement.

The somatic response to the attempt to penetrate the anal canal is contraction of the external anal sphincter and defensive lowering of the tail, but, whenever the canal is entered, somatic expulsive efforts set in.

In lightly anaesthetized preparations the somatic expulsive response consists of elevation of the tail, of contraction of the abdominal muscles, of stretching and then straddling of the hind limbs. Finally, on cessation

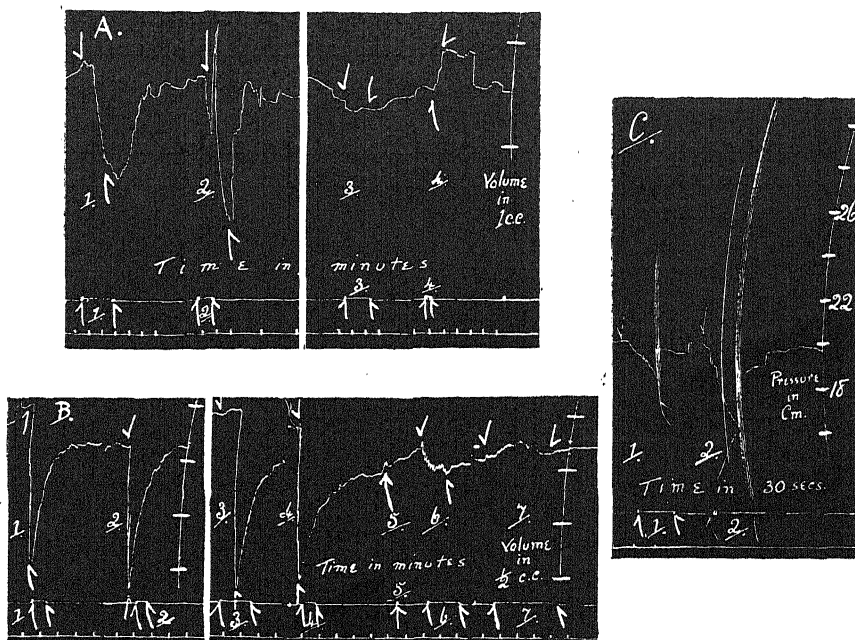


Fig. 3.

- A. Cat: decerebrate; lumbar outflow cut; pudendal nerves cut; volume record from anal canal at constant pressure of 21 cm. H₂O. (1) Balloon within large bowel rotated. (2) Balloon within anal canal rotated. (3) Pelvic nerves cut. Balloon within large bowel rotated. (4) Pelvic nerves cut. Balloon within anal canal rotated.
- B. Cat: decerebrate; pudendal nerves cut; lumbar outflow cut; lumbo-sacral cord isolated; volume record from anal canal at constant pressure of 21 cm. H₂O. (1) Balloon moved to and fro within large bowel. (2) Balloon rotated within anal canal. (3) Full curarization induced. Balloon moved to and fro within large bowel. (4) Balloon rotated within anal canal. (5) Spinal anaesthesia induced. (6) Balloon rotated within anal canal. (7) Balloon moved to and fro within large bowel.
- C. Cat: decapitate; pressure record from anal canal at constant volume. (1) Balloon within large bowel rotated. (2) Lumbar outflow cut. Balloon within large bowel rotated.

of the stimulus, twitching in the external anal sphincter and in the pelvocaual muscles sets in. In decapitate preparations the somatic response is less marked and less purposeful, but twitching in the external

anal sphincter is most obvious. Indeed, as Ott [1879] showed, rhythmical twitching in the external anal sphincter is a striking phenomenon in cats with transection of the spinal cord.

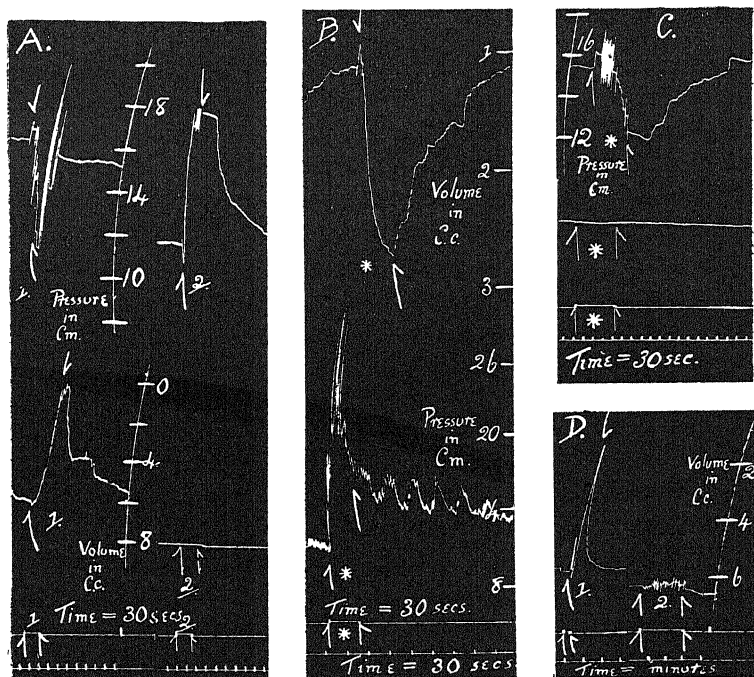


Fig. 4.

- A. Cat: anaesthesia Dial Liq. Compound—"Ciba"—0.5 c.c./kg.; upper pressure record from anal canal at constant volume; lower volume record from large bowel at constant pressure of 18 cm. H_2O . (1) Balloon in anal canal rotated. (2) 5 p.c. cocaine HCl applied to mucous membrane of anal canal. Balloon in anal canal rotated.
- B. Cat: Na amytal anaesthesia—50 mg./kg.—upper volume record from anal canal at constant pressure of 33 cm. H_2O ; lower pressure record from large bowel at constant volume. * = Balloon in large bowel rotated.
- C. Cat: decerebrate; pelvic nerves cut; upper pressure record from anal canal at constant volume; lower volume record from large bowel at constant pressure of 20 cm. H_2O . * = Balloon within anal canal rotated.
- D. Cat: decerebrate; volume record from large bowel at constant pressure of 21 cm. H_2O . (1) Rotation of balloon within large bowel. (2) Pelvic nerves cut. Rotation of balloon within large bowel.

When the cord is cut in the lower thoracic region, the somatic response consists of flexion of the caudal limbs onto the abdomen and of contraction of the abdominal muscles. The tail is not elevated.

The essential local dilatation of the anal canal may be obtained in anaesthetized cats, in decerebrate cats, in decapitate cats and in cats with isolated lumbo-sacral cord (Fig. 3 A(2), B(2); Fig. 4 A(1)).

The response is independent of the integrity of the pudendal nerves (Fig. 3 A, B), is enhanced by division of the lumbar outflow and full curarization does not affect the response (Fig. 3 B(4)).

After division of the pelvic nerves (Fig. 3 A(4)), and after induction of spinal anaesthesia (Fig. 3 B(6)), stimulation of the anal canal does not lead to dilatation of the anal canal. Infrequently dilatation of the anal canal was obtained, after section of the pelvic nerves, in the presence of intact pudendal nerves (Fig. 4 C).

Application of 5 p.c. cocaine hydrochloride to the mucous membrane of the anal canal causes sustained dilatation of the canal, and stimulation may now lead to constriction of the canal (Fig. 4 A(2)).

(4) *The response of the large bowel to stimulation of the anal canal.*

Movement of the balloon in the anal canal leads to contraction of the large bowel in anaesthetized cats (Fig. 4 A(1)), in decerebrate and in decapitate cats (Fig. 5 A) and in cats after transection of the cord in the lower thoracic region (Fig. 2 C(1)). Shortly after decapitation, and less often after decerebration, stimulation of the anal canal frequently leads to relaxation of the large bowel (Fig. 5 D). Very vigorous stimulation may superimpose contraction on this initial relaxation. Such a relaxation response invariably gives place, later in the experiment, to contraction, while division of the lumbar outflow immediately converts the relaxation into contraction. Such relaxation has not been obtained in the presence of an intact lumbar outflow after division of the pelvic nerves, but in one cat, with both outflows intact, stimulation of the anal canal, after induction of spinal anaesthesia, led to relaxation of the large bowel.

The usual motor response is not affected by division of the pudendal nerves (Fig. 2), is facilitated and augmented by section of the lumbar outflow (Fig. 5 A(1, 2)) and occurs during full curarization (Fig. 2 D(1)).

After induction of spinal anaesthesia (Fig. 2 E(2)), and after cutting the pelvic nerves (Fig. 5 A(4)) stimulation of the anal canal is without effect on the large bowel.

If the pelvic nerves are cut and the pudendal nerves are intact, movement of the balloon within the anal canal may cause undoubted hardening of the abdominal muscles and some attempt at adoption of a defaecation

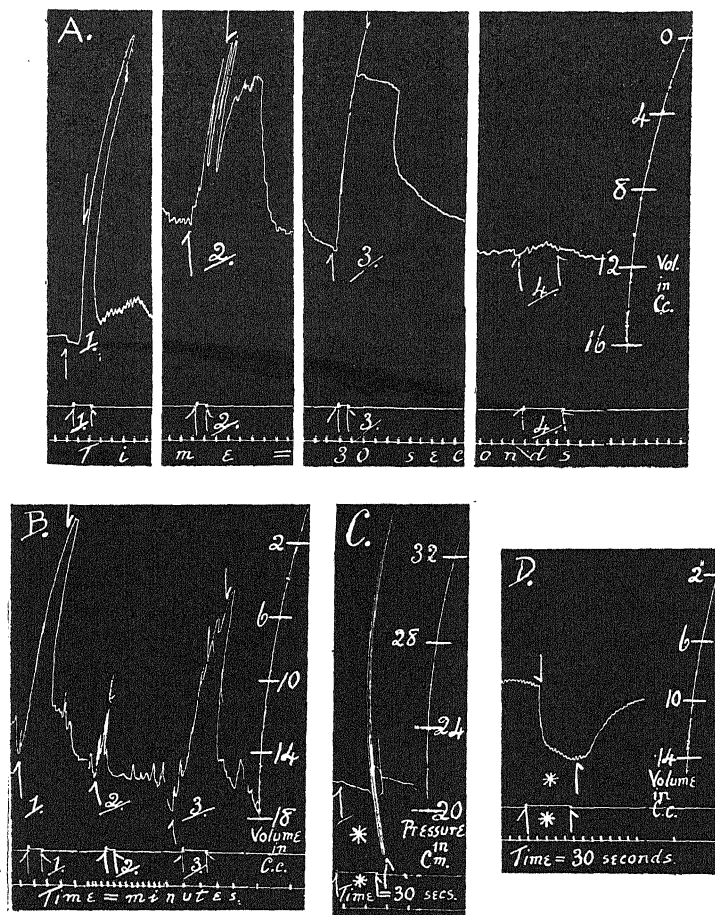


Fig. 5.

- A. Cat: decerebrate; volume record from large bowel at constant pressure of 20 cm. H₂O. (1) Balloon within anal canal moved to and fro. (2) Lumbar outflow cut. Stimulus applied as before. (3) Pelvic nerves exposed. Stimulus applied as before. (4) Pelvic nerves cut. Stimulus applied as before.
- B. Cat: decerebrate; lumbar outflow cut; lumbo-sacral cord isolated; volume record from large bowel *via* colostomy at constant pressure of 21 cm. H₂O. (1) Balloon in large bowel moved to and fro. (2) Spinal anaesthesia induced. Stimulus applied as before. (3) 90 min. later. Stimulus applied as before.
- C. Cat: decapitate; lumbar outflow cut; anal canal deprived of visceral innervation by gut section; pressure record at constant volume from anal canal. * = Balloon within cranial segment of gut rotated.
- D. Cat: decerebrate; volume record from large bowel at constant pressure of 23 cm. H₂O. * = 60 min. after decerebration balloon within anal canal rotated.

posture. Such responses have never been vigorous and have had no marked influence on the balloon in the large bowel.

Application of 5 p.c. cocaine hydrochloride to the mucous membrane of the anal canal abolishes the response from the large bowel (Fig. 4 A (2)).

The effect of division of the large bowel on the responses.

Unfortunately it is not possible to cut the large bowel and to leave intact the innervation to both the cranial and caudal segments of the gut produced by the division. It is possible, however, so to cut the large bowel that the cranial segment retains the lumbar and sacral visceral outflows, while the caudal segment, deprived of its visceral innervation, yet retains the somatic nerve supply by the pudendal nerves to the external anal sphincter and to the circumanal region. To do this the gut must be cut just caudad to the peritoneal reflection, with the result that the caudal segment, including the anal canal, is very short.

Such division of the large bowel, which necessitates splitting the symphysis pubis, may be carried out by section between ligatures, in which case the balloon for the cranial segment must be introduced through a colostomy, or the bowel may be tied on to a short length of flanged glass tubing of bore sufficiently wide to allow free movement for the holder of the balloon in the cranial segment. The latter method is simpler, preserves the integrity of the peritoneal cavity, and the continuity of the muscular tissues of the gut wall and of the visceral nerves running on the surface of the gut is effectively interrupted by the ligature. Two experiments were carried out by the former, four by the latter method.

After such division the response of the large bowel to stimulation of the large bowel was unaffected. Stimulation of the large bowel cranial to the point of section, on the other hand, no longer produced unequivocal dilatation of the anal canal, although, so long as the pudendal nerves were intact, such stimulation still led to post-stimulation twitching in the external anal sphincter (Fig. 5 C).

Even when the pudendal nerves were intact, stimulation of the anal canal led in no case to relaxation of the anal sphincters, and in only two of the six cats did contraction of the large bowel result. So long as the pudendal nerves were intact stimulation of the anal canal led to some response from the abdominal muscles.

In two cats the colon was divided between ligatures just caudad to the inferior mesenteric artery. In these cases the cranial segment of the large bowel received only the lumbar outflow by the lumbar colonic

nerves, while the caudal segment received the sacral outflow and the hypogastric nerves. The recording balloon for the cranial segment was passed into the gut through a colostomy close to the ileo-cæcal sphincter. The caudal segment responded to stimulation in normal fashion, but no effect was transmitted to the cranial segment. Stimulation of the cranial segment produced no response locally and was without influence on the caudal segment.

DISCUSSION AND CONCLUSIONS.

The nature of the stimulus for defæcation.

Even if it be conceded, in spite of Lehmann's work [1913], that the afferent impulses eliciting defæcation arise locally in the caudal part of the gut, there are difficulties in accepting the method employed in the present series of experiments as representative of a normal mode of eliciting defæcation.

Zimmermann [1909], experimenting on himself, found that the rectal mucous membrane 10 to 12 cm. craniad to the anus is insensitive to touch, to moderate variation in temperature and to electrical stimulation. Nevertheless, when the rod used to test for tactile sensation caught on a fold of mucous membrane, an unpleasant dragging sensation was produced. As the anal canal was approached, temperature sensation appeared and stimulation with an electrical current was felt. Distension of the rectum by means of a balloon at a pressure of 20 mm. Hg produced a desire to defæcate. Variations of 2 to 3 mm. Hg pressure could be detected.

Hertz [1911] also found that the rectal mucous membrane in man is insensitive to tactile, to painful and to thermal stimuli, while the mucous membrane of the anal canal is sensitive to thermal stimuli and to friction. Distension of the rectum gives rise to the sensation of fullness associated with the desire to defæcate. The closer to the anal canal, the greater the length of the distending balloon and the more rapid the distension, the less the pressure necessary to evoke the sensation.

Distension of the large bowel is supposed to act upon sense organs in the muscles of the gut wall. The inadequacy of distension as a stimulus in the present series of experiments is striking. This may be due to the acuteness of the experiments and consequent shock to the nervous system. Under such conditions a gross stimulus such as movement within the gut, with inevitable dragging on the wall of the viscus, may be necessary to reach the threshold for the reflex.

Nevertheless, there is some evidence that sensitive tissues are placed nearer the lumen of the gut than the muscle coats. The disappearance of all response on cocainizing the mucous membrane is suggestive. The cocaine, of course, may penetrate and affect sensory endings deep to the mucous membrane. Again the rectal tenesmus which occurs in severe diarrhoea, when there is practically no material in the gut apart from a small quantity of thin ichorous fluid, is difficult to explain if distension is the sole stimulus.

While distension is an adequate stimulus for peristalsis in the small intestine [Trendelenburg, 1917], it is doubtful if this applies in the same degree to the large intestine [Alvarez, 1929]. Cannon [1912] also comes to the conclusion that the cause of true peristalsis in the caudal region of the large bowel must be sought for in a stimulus other than pure distension, and he suggests that movement of firm faecal masses may supply this stimulus.

It may be, then, that the mucous membrane of the large bowel, although insensitive to localized contact, is yet sensitive to gross movement such as that produced by the passage of firm faecal masses into the caudal region of the gut. Such a conception would explain most effectively the transitory nature of the "call to defaecation" in man.

There is less difficulty in accepting movement within the anal canal as a normal stimulus. Such a conception is in keeping with the experimental work on man. In addition, Barrington [1921] used movement of a catheter or of fluid within the urethra to elicit micturition responses in the cat. Such susceptibility of the mucous membrane of the anal canal to a frictional stimulus, however, is obviously designed to maintain a defaecation effort already in progress rather than to initiate such an effort.

The response of the large bowel to stimulation of the large bowel.

This response is largely dependent on the lumbo-sacral region of the cord. Nevertheless, the gut seems to retain some degree of autonomy; possibly in preparations surviving destruction of the lumbo-sacral cord this autonomous power is enhanced, or, probably more correctly, regains much of its primitive independence.

The lumbar outflow is inhibitory and both afferent and efferent pathways are in the pelvic nerves.

The response of the urinary bladder to distension likewise depends on the integrity of the pelvic nerves, but this response, far from showing indications of autonomy, depends on a micturition centre in the mid-brain [Barrington, 1928]. However, the presence of a centre for the

large bowel in the lumbo-sacral cord is in keeping with the results of Head and Riddoch [1917] and of Riddoch [1917] in spinal man. In such patients the evacuation of the gut, and also of the bladder, seems to be complete.

The response of the anal canal to stimulation of the large bowel.

This response is also largely dependent on the integrity of the pelvic nerves and of the lumbo-sacral cord, and the lumbar outflow exerts an inhibitory influence.

It is difficult to determine the part played by the pudendal nerves and by the striped anal sphincter. Dilatation of the anal canal occurs in all cases after division of the pudendal nerves, while indication of participation of the pudendal nerves in the response is shown by the post-stimulation anal twitching which is present on stimulation of the large bowel after gut section. Unfortunately, to eliminate participation of the internal anal sphincter without gut section necessitates division of the pelvic nerves and destruction of the afferent pathway for the response; failure to show relaxation of the external sphincter after gut section may be due to difficulties of recording in such cases. Ott [1879], however, has shown that inhibitory impulses reach the external anal sphincter from a centre in the brain, so that it is possible that normal relaxation of the external anal sphincter in defæcation can occur only in intact animals. Nevertheless, Barrington [1921, 1928] finds that dilatation of the urethra, as a result of distension of the bladder, depends on a centre in the lumbo-sacral cord, the afferent pathway being in the pelvic nerves and the efferent pathway in the pudendal nerves.

The response of the anal canal to stimulation of the anal canal.

This response also, so far as the internal anal sphincter is concerned, is dependent on the lumbo-sacral cord alone and on the integrity of the pelvic nerves. The lumbar outflow again tends to inhibit the response.

The dilatation obtained after section of the pelvic nerves in decerebrate cats is not a constant response. Probably the relaxation of the external anal sphincter under such conditions is part of a general somatic response which certainly can be elicited, although imperfectly, by stimulation of the anal canal after division of the pelvic nerves. Barrington [1921] finds that the reflex relaxation of the urethra, which occurs on passage of fluid through the urethra, is controlled by a centre in the lumbo-sacral cord, and has both afferent and efferent channels in the pudendal nerves.

The constriction of the anal canal produced by stimulation after cocainizing the mucous membrane may be the normal contraction response of the circumanal tissues during an attempt to penetrate the anal canal. Such contraction is probably due to stimulation of subcutaneous receptors protected from the cocaine, while the submucous receptors, stimulation of which produces relaxation of the anal canal, are anaesthetized.

The response of the large bowel to stimulation of the anal canal.

The efferent pathway for this response is in the pelvic nerves. After division of the pudendal nerves the response persists so that the pelvic nerves also serve as an afferent path. Probably, however, the pudendal nerves, too, transmit centripetal impulses capable of eliciting contraction of the large bowel, just as afferent impulses from the urethra along the pudendal nerves elicit contraction of the bladder [Barrington, 1921]. The centre controlling this second micturition reflex is situated in the mid-brain, while the centre for the corresponding gut response is in the lumbo-sacral cord. This finding for the gut is in keeping with the results of most workers who find apparently normal defæcation in spinal mammals [Sherrington, 1900].

The influence of the lumbar outflow is once more inhibitory.

Barrington [1921] has shown that stimulation of the urethra causes contraction of the urinary bladder through a reflex arc passing by the hypogastric nerves and a centre in the lumbo-sacral cord. A corresponding response does not appear in the gut. Probably this is due to the fact that part of the bladder musculature in the cat receives a motor nerve supply from the lumbar outflow [Elliott, 1906]. This state of affairs, so far as is known, does not hold in the case of the large bowel.

The nervous connections for the relaxation of the large bowel which frequently occurs on stimulation of the anal canal shortly after decapitation and less frequently after decerebration are obscure. Possibly this is the usual response to noxious stimuli unmasked by temporary depression of the more sensitive expulsive responses. On the other hand the response may be of the nature of a Sokownin reflex through the inferior mesenteric ganglia.

The responses in general.

At the caudal end of the alimentary tract the central nervous system no longer exerts merely a general diffuse influence on the gut, but takes an active part in reception of stimuli from and in emission of impulses

to the gut. This more intimate relationship of the spinal cord to the gut seems to be confined to the sacral outflow, the lumbar outflow exerting the usual "sympathetic" restraining influence on the translation of bowel contents. However, the gut seems not to have wholly surrendered its independence because stimulation of the large bowel is still able, after destruction of the lumbo-sacral cord, to cause contraction of the large bowel and dilatation of the anal canal. In this way it is possible to explain the findings of Gowers [1877] in the patient with injury to the sacral roots, of Goltz and Ewald [1896] in their cordless dogs, of Bayliss and Starling [1900], of Elliott and Barclay-Smith [1904] and of Barrington [1915].

The pathways in the pudendal nerves seem to be much less important in the defæcation responses than in the micturition reflexes. Probably the pudendal nerves do play a part in defæcation, but the difficulties in the way of satisfactory demonstration of this fact are great. On the other hand, it is possible that Barrington's technique recorded mainly the responses of the external sphincter urethræ innervated by the pudendal nerves, so that the full rôle of the pelvic nerves in micturition escaped notice.

The somatic defæcation responses, which are most elaborate, seem to depend on centres cranial to the lumbo-sacral cord, and probably for their full expression, cranial to the spinal cord itself.

SUMMARY.

The behaviour of the caudal end of the gut is recorded by two tandem balloons usually inserted through the anus. Movement of the balloons themselves within the large bowel or within the anal canal elicits co-ordinated expulsive efforts from the gut and from the somatic musculature. Simple distension of the balloons within the gut or within the anal canal is not an adequate stimulus in acute experiments. The responses persist after complete curarization of the somatic musculature, and application of cocaine to the mucous membrane of the large bowel and of the anal canal abolishes all response.

The visceral responses persist after transection of the spinal cord in the lower thoracic region and depend mainly on the integrity of the pelvic nerves. The lumbar outflow exerts an inhibitory influence on all the responses.

Four main responses are present: (1) Stimulation of the caudal end of the large bowel causes contraction of the large bowel: (2) Relaxation of the anal canal. Both these responses may appear infrequently after

complete decentralization of the gut. (3) Stimulation of the anal canal causes dilatation of the anal canal. This dilatation is due mainly to relaxation of the internal anal sphincter. When the pudendal nerves are intact, it is probable that the external anal sphincter participates in the response. (4) Stimulation of the anal canal causes contraction of the large bowel. The main afferent pathway is in the pelvic nerves, but the pudendal nerves probably also convey centripetal impulses for this response.

Shortly after interference with the cerebro-spinal axis, stimulation of the anal canal causes relaxation of the large bowel. The pathways for this response are obscure. The findings are discussed and compared with the corresponding responses of the urinary tract.

I am deeply indebted to Prof. Cathcart for constant encouragement.

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ACTION POTENTIALS IN SYMPATHETIC NERVES,
ELICITED BY STIMULATION OF
FROG'S VISCERA.

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THE sensory impulses elicited by stimulation of the frog's skin have been the object of repeated study by Adrian [1926, 1932] and his collaborators, Zotterman [1926], and Cattell and Hoagland [1931]. These impulses fall into several groups, characterized by different modes of excitation and different rates of conduction. In one group are impulses seemingly from tactile endings, excited by light touch with cotton wool or by an interrupted jet of air. Such impulses are rapidly conducted (Matthews [1929] found an order of 14 m. per sec. at 14° C.), and the action potentials developed are relatively large. This group of sensory impulses is assigned, with fair certainty, to the moderately large medullated component of cutaneous nerves. In Erlanger and Gasser's classification [1930] they would come within the lower reaches of group A. On the other hand, procedures designed to injure the skin, such as scraping, crushing, burning, or the application of acid, evoke trains of slowly conducted impulses—impulses often travelling less than 1 m. per sec. The action potentials of this group are correspondingly small, and some of them may be allotted [Adrian, 1931 *b*] to non-medullated nerve fibres of Erlanger and Gasser's group C. The mode of excitation of these impulses allows them a rôle in the mediation of pain, though, considering their slow conduction, by no means the totality of this function. A third group with an intermediate conduction rate is produced by moderate heat [Adrian, 1932 *a*]. The present work set out to survey, in a similar manner, the impulses which could be excited in sympathetic nerves by the ordinary types of stimulation applied to viscera, with a view to comparing the range of visceral sensory activity with the somatic sensory activity previously studied.

METHODS.

A series of exploratory experiments employed pithed but otherwise intact frogs (*Rana temporaria*), but routinely, an excised preparation of the viscera was used, either spread out on filter paper moistened with Ringer's fluid, or suspended in a bath of Ringer's or in air. To make the preparation, the sympathetic rami communicantes V to XI were cut at their junction with the spinal nerves, and the long intercommunicating ramus between the ganglia V and IV, at its cephalic end. Ligatures were first placed on the nerves required for leads. The gut was then tightly tied off and cut above the stomach and below the rectum, and the vascular and peritoneal connections from the viscera to the body wall freed. The simplest preparation consisted of the gastrointestinal tract and pancreas, with the attached mesentery containing in its root the spleen. Sometimes only the stomach and its mesentery, or the intestine and its mesentery were taken. Again, preparations were made to contain other organs, the liver, the kidneys, the reproductive tract. In the end the investigation covered all the viscera with the exception of the heart and lungs.

For electrode leads the rami communicantes were used, white and grey together, or the intercommunicating ramus V to IV. This latter afforded the longest and slenderest and most satisfactory lead. Each lead contained pre-ganglionic and post-ganglionic efferent fibres, besides the sensory fibres under investigation. Histological examination of a number of specimens showed that the rami frequently also contained nerve cells, for in the frog the cells of the sympathetic ganglia are not closely aggregated. This complexity of the lead must be borne in mind in interpreting the records.

The set-up and technique used for recording the nerve impulses need only brief description. Electrodes of the silver-silver chloride variety led from reservoirs of Ringer's fluid. The nerve rested on threads projecting from the reservoirs. Amplification was supplied by four valves in cascade, with resistance-capacity coupling, leading to four pentode power valves arranged in parallel to supply the coils of a Matthews oscillograph. In the first three stages large condensers, $4\mu\text{F}$, were used, but at the output stage a $2\mu\text{F}$ condenser was substituted. 0.5 megohm grid leaks were used throughout. The beam of light from the oscillograph was split and made to record both on a viewing screen, and in a slit camera, as described by Adrian, Cattell and Hoagland [1931]. The bromide paper passed the camera slit at the rate of 20 cm. per sec., while the

image thrown by the rotating mirror on to the viewing screen travelled 2 m. per sec.—ten times faster. The amplified potential was also tapped, and made to operate a loud-speaker, by means of an auxiliary amplifier and power tube. Thus the impulses were heard, as well as seen and photographed.

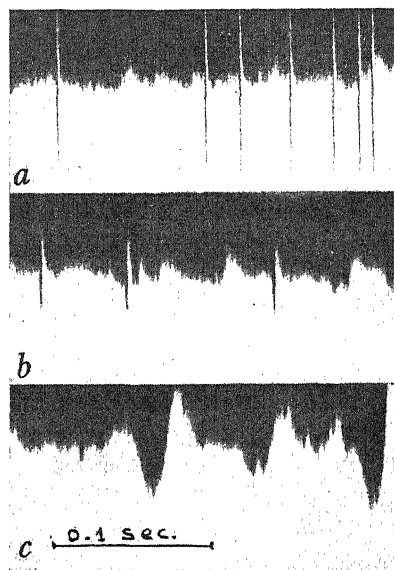
For ordinary purposes the amplification used was fairly constant, a potential difference of 50 microvolts at the input producing a deflection of the order of 1.2 cm. on the photographic film. All the records chosen for reproduction conform to this sensitivity. Thus the larger deflections, the so-called fast impulses, represent potentials between 50 and 100 microvolts, while the slow impulses fall below this first figure.

In reading the records (left to right) an initial downward deflection indicates relative negativity at the distal earthed electrode. The ascending sensory impulses under investigation were all in this category. Descending impulses appeared in some preparations. The first impulse of Plate II *d* is of this sort. Such descending impulses may represent either discharges in injured or hyper-irritable nerve fibres, or spontaneous activity of isolated ganglion cells included in the lead. Drops falling from the insulated table on which the preparation was mounted produced deflections in the sense of initial negativity at the proximal or input electrode, not followed by positivity. These are not to be confused with descending impulses. Thus in Plate II such deflections have been eliminated between *c* and *d* by the simple expedient of mopping up the excess fluid. The very small oscillations on the base line of all the records originated within the amplifier, not in the preparation. They set a lower limit, of the order of a few microvolts, to the potentials which the set-up was capable of demonstrating.

Spontaneous activity in the sympathetic rami introduced a complication into the records. This activity so resembled that evoked by the application of nicotine or a crystal of salt locally to a ganglion, that it may be laid chiefly to impulses travelling in post-ganglionic sympathetic efferent fibres. During the breeding season the spontaneous activity sometimes reached the proportions of a general seizure, all the cells seemingly discharging repeatedly for a matter of minutes and throwing the gastrointestinal tract into spasm. The act of pithing the frog appeared to render the ganglia irritable, for their stability was greatly increased by leaving the animal, circulation active, for an hour or more after pithing and before making the preparation. As the breeding season passed, the ganglia became more stable. Yet even in the quietest preparations a "control" run for a few seconds always showed occasional small impulses or waves.

OBSERVATIONS.

The action potentials which were led off from the frog's sympathetic rami and trunk during and after stimulation of the viscera, formed a complete series. This series was, however, dominated by three types of potential which may, for convenience, be designated fast impulse, slow impulse and waves. Text-fig. 1 shows examples of each of these.



Text-fig. 1. Fast and slow impulses and waves. Isolated preparation of frog's gastrointestinal tract spread on filter paper; electrodes 4 mm. apart on right ramus intercommunicans V to IV.

a, fast impulses; touch applied to mesentery of stomach. *b*, slow impulses; pressure applied to body of stomach. *c*, waves; pressure on stomach maintained.

Fast impulses of large potential.

If a preparation of the frog's viscera be touched or brushed or mechanically deformed in any way, an outburst of large, rapidly conducted impulses is the conspicuous result. Plate I *a* and *b* show two such discharges obtained from different parts of the stomach mesentery of the same frog. The one consists of very large action potentials of high velocity, the other of potentials smaller and probably slower. Fast impulses were excitable in greatest number from the mesentery. They

have been obtained from the pancreas, spleen and kidneys, probably from the peritoneal coverings of these organs. The liver yielded them only along the line of mesenteric attachment. Mechanical stimulation of the hollow viscera either by touch or pressure applied from without, or from within, by inflation, did not produce them unless the mesentery was also disturbed. Neither the viscus itself nor its serosa seemed, in these organs, to be supplied with the nerves and endings giving rise to the "fast impulse."

The mechanism serving the fast impulses appeared to be most densely distributed along the course of the blood vessels radiating from the root of the mesentery, and more densely near the root of the mesentery than near its periphery. By directing a fine blunt glass point held in a rack and pinion, under a binocular microscope, this concentration along the blood vessels was confirmed. Further, it was established that large areas of the mesentery lying between the major blood vessels were quite free of the activity. The distribution was not spot-like, in the order of magnitude of the skin spots studied by von Frey. On the contrary, a whole sector of radiating blood vessels appeared equally able to excite probably the same fast fibre. On a microscopic scale, the distribution might still be one of spots, but this could not be determined.

Although these impulses were evoked by light touch, tension directed along the line of the blood vessels was a far more efficacious stimulus. The character of the endings as tension receptors was better shown when the preparation was suspended in a bath of Ringer. Under the fluid, the surface of the mesentery could be touched, even rubbed with a glass rod without yielding impulses. But the slightest strain directed along the blood vessels, especially if transmitted to the root of the mesentery, excited an outburst.

The size and velocity of these "fast impulses" indicate that they are travelling in fair sized medullated nerve fibres. In osmic acid preparations medullated fibres, 6-7 μ in diameter, were found in limited number in sections of the upper rami used for leads. In a Vth ramus one fibre of diameter 10 μ was seen. The majority of the medullated fibres in all the rami and all the medullated fibres in the IXth ramus did not, however, exceed 3 μ . When search was made in a methylene blue preparation of the frog's mesentery (*Rana pipiens*) stained by Dr Hines, small medullated fibres were found along the blood vessels. These were quite numerous, often forming small trunks in which they branched repeatedly. Sometimes the branches were unmyelinated. Yet endings more complicated than a simple unencapsulated knob could not be found, and even

these were exceedingly rare. The difference of species may, however, be significant in this result.

Experiments were made on decerebrate and spinal frogs to determine the reactions of the animal to stimulation aimed at the fast sensory mechanism. The viscera were exposed through a slit in the abdominal wall and the vessels stroked with a fine glass rod. The animal gave no visible response. Slight tension was equally disregarded. Only when the stimulation exceeded that which would excite the fast impulses alone were skeletal and visceral reflexes evoked. A physiological stimulus to the mechanism was also sought in vain. Movement of peritoneal surfaces on one another was not effective. The impulses were not called out by even the most violent spasms of the gut occurring spontaneously or excited by nicotine. In jumping, the drag of the viscera on the root of the mesentery may excite the mechanism, and some important reflex adjustment be involved, but this possibility was not open to experimentation. The significance of these endings and impulses in the physiology of the intact animal remains, then, obscure.

Slow impulses of smaller potential.

Among the large, fast impulses evoked by touching the mesentery, occasional smaller, slower impulses were seen. Plate I *b* shows some of these. Exploration of the mesentery demonstrated that these slow impulses were much more widely excitable than the fast variety; indeed, that a slow mechanism was quite ubiquitous. But the light touch or slight tension which was the most adequate stimulus for the fast impulses, provoked only an occasional example of the slow variety. When, however, touch was increased to pressure, and more than momentarily maintained, trains of the slow impulses were set up. Further study of their mode of excitation showed that any procedure which could be classed as injurious to the tissue, pinching, scraping, crushing, burning with acid, gave rise to slow impulses, with or without the fast variety.

Two procedures were settled upon as most reliable in the production of slow impulses—pressure approaching the crushing point and burning with acid. The pressure was applied through a blunt glass point, either by hand or with a rack and pinion. The acid, 25 p.c. acetic or hydrochloric, was applied locally by means of a tiny cotton pledget, and into the lumen of the gastro-intestinal tract by cannula. Using these two agents, a survey was made of the distribution in the periphery of the slow sensory mechanism. Slow impulses were recorded from all the abdominal viscera investigated except the testis, ovary and oviduct.

They could be obtained with great ease from the entire mesentery, and from areas between the blood vessels not supplied with the fast mechanism. The mesentery of the pyloric angle was particularly rich in this activity. The entire gut yielded the slow impulses, but again, in greatest number from the pyloric region. Acid was effective, applied either to mucosa or to serosa, but from the mucosa the impulses were fewer and smaller. The parenchyma of organs such as the liver and kidney seemed to give rise to the impulses, though again in lesser number than their peritoneal coverings. The procedures resorted to to excite the slow impulses precluded any rigorous study of spot distribution. There was, however, no indication of any such arrangement.

The action potentials excited by these more or less destructive procedures differed enormously from place to place, and even from the same place during continued stimulation. On the whole, the impulses from the mesentery tended to be faster and larger than those from the gut, while the impulses from the parenchyma of liver and kidney were the smallest of all. But exceedingly small impulses appeared occasionally during stimulation of the mesentery, and in numbers, from the gut. In general, also, the more destructive and longer sustained the stimulation, the smaller and slower were the impulses recorded. Thus the impulses of Text-fig. 1 *b*, which are the fastest of the slow type, were the first impulses excited as pressure was applied to the pylorus. Plate I *c* and *e* show the discharge, as pressure was maintained, of progressively slower impulses. An exceedingly slow variety, shown in Plate II *b* and *f*, was often obtained together with the faster sorts, as the result of the action of acids. During sustained pressure stimulation, a more rapid adaptation of the faster mechanisms seemed to be the factor in the changing character of the impulses discharged. The mechanism giving the very slow type of impulse adapted only very slowly, after many seconds. With acid, however, a differential resistance of the several types of nerve fibre to the ultimately lethal action of the stimulant produced the same picture.

Search was made in the methylene blue stained preparation of mesentery for nerves and endings in the region between the blood vessels, the regions yielding the slow but not the fast impulses. Nerves were present in confusing number, forming a plexus of small trunks and single fibres most of which could be traced from the root of the mesentery to the gut. Non-myelinated and finely myelinated fibres were intermingled in this plexus. Of endings, nothing was found more than a very occasional free terminal.

The service of this system of slow sensory impulses to the animal as a whole was investigated in the series of decerebrate and spinal frogs. The procedures used to evoke slow impulses (pinching, scraping, acid) excited both skeletal and visceral reflexes. Strong stimulation produced shuffling and jumping movements, the jumping tending to predominate when the stimulus was caudally applied, as for example, to the rectum, and shuffling when the stimulation was more cephalad. But increasing the stimulation in an active preparation always converted shuffling to jumping. Weaker stimulation produced reflex contractions of back or flank musculature. The secretion of mucus by the skin was also conspicuously increased. Visceral reflexes evoked, included inhibition of respiration, an almost invariable result, and slowing of the heart. A long latency, often several seconds, characterized both skeletal and visceral reflexes, more especially the former.

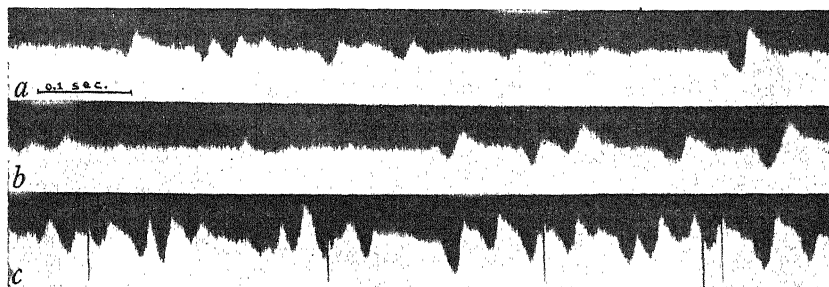
The slow impulse mechanism would seem, then, to bring into play all the reactions usually associated with pain sensation. Whether all or only a part of the range of moderately slow, slow and very slow impulses is responsible for these reactions is not known. Further study with finer methods of stimulation may show that such very different types of impulse as those of Text-fig. 1 *b* and those of Plate I *c* and *f* represent quite different sensory functions. Certainly the moderately slow and very slow varieties must be travelling in different types of nerve fibre. The former would seem to belong in Heinbecker's [1930] group B 2, or at almost B 3. The latter, surely, from their similarity with the visceral efferent discharge, come within group C, though Heinbecker himself found no afferent fibres belonging to this group in the sympathetic nerves which he examined. However, until further work shall sort out this catch-all of slow impulses, it may be considered a system of visceral sensibility of an indeterminate but protopathic type.

Waves.

Action potentials so slow in developing and in subsiding, and so variable in magnitude that they could not be considered impulses, were a prominent feature of the records. They were given the designation waves. The waves attained their maximum development during stimulations designed to evoke the slow type of sensory impulse. But the light touch provocative of a train of fast impulses was also sometimes followed by a suggestion of this activity. Some of the most perfectly formed examples of waves were seen in "control" records (Text-fig. 2 *a*), and almost all the control records showed traces of them. Indeed, waves

were a constant element in all spontaneous activity, as well as in the discharges provoked by mechanical or drug stimulation of the sympathetic ganglia.

In form, the smaller and more discrete waves (Text-figs. 1 *c* and 2 *a*) represent a process of relative negativity at the distal electrode. Such waves were usually diphasic, the phases bearing the same relationship as in an ascending impulse. The small waves of control records exhibited a certain uniformity from preparation to preparation, a duration of the positive phase of the order of 0.02–0.03 sec. and a tendency to appear in groups. Such grouped discharge was often in crescendo, and followed by a pause.



Text-fig. 2. Preparation of gastro-intestinal tract suspended in a bath of Ringer's, cannulae in oesophagus and rectum; electrodes 3 mm. apart on right ramus intercommunicans V to IV.

a, control, showing the largest waves developed. *b*, immediately after inflation with Ringer's, showing beginning augmentation of the waves. *c*, maximum development of waves; impulse discharge in two fibres, one monophasic, the other diphasic.

Waves which were not spontaneous, but the product of sensory stimulation, showed a direct proportionality in frequency, magnitude and duration to the intensity of the stimulating procedure, to the amount of pressure or the concentration of acid. The extent of the area under stimulation also influenced the size of the response. Thus, the very largest waves were obtained when acid was caused to flow through the entire gastro-intestinal tract. Inflation of the tract with air or Ringer's fluid produced waves almost as large. The waves evoked by local applications of pressure or acid were, on the whole, considerably smaller.

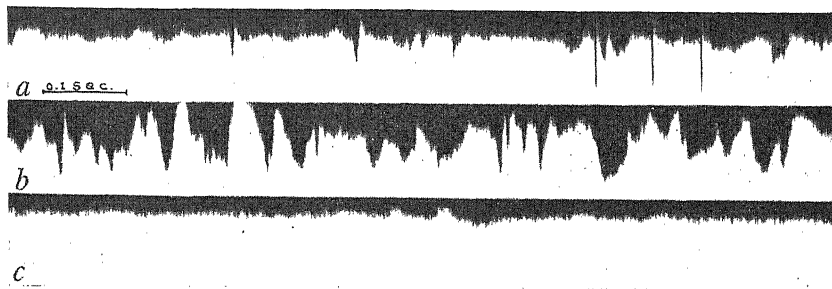
The waves developed during stimulation tended to conform at first to the pattern of the spontaneous waves, amplitude and frequency alone being augmented. The effect of mechanical stimulation shown in Text-fig. 2 was of this nature. But prolonged or excessive stimulation of an

active preparation always increased the duration of the waves, together with their amplitude, and the original simplicity of form was lost in a rapid succession of interfering potentials. The picture was further complicated in most experiments by impulse discharge. Such were the reactions to acid stimulation of Plate II and Text-figs. 3, 4. At the height of a discharge, however, the effect on duration was always less than the effect on amplitude and frequency. The latter were multiplied many fold, the first rarely more than doubled. In this connection, distortion imposed by the condenser coupling in the amplifier may have had a part in curtailing the longest of the waves, but on waves of duration less than 0.05 sec., which includes the greater part of the wave activity, this effect was negligible.

The waves also bore definite relations in time to the stimulating procedure. A certain time, $1/8$ to $1/2$ sec., was required for the waves to appear, and a much longer time, often several seconds, for their maximum development. The more intense the stimulation, the shorter were both these intervals. Again, on cessation of stimulation the waves subsided, and rather more rapidly than they were originally built up. A certain amount of adaptation was exhibited by the wave mechanism. When a locally applied pressure was maintained for minutes, the waves reached a maximum and then diminished, but rarely ceased entirely. The failure of complete adaptation was shown in the many experiments in which the gastro-intestinal tract was inflated with air or Ringer's fluid, and could not again be deflated. Wave discharge continued, under these conditions, often for as long as the preparation was observed, in one case for 2 hours. The discharge was not, however, constant, but showed in exaggerated form the tendency to grouping noted in the control records.

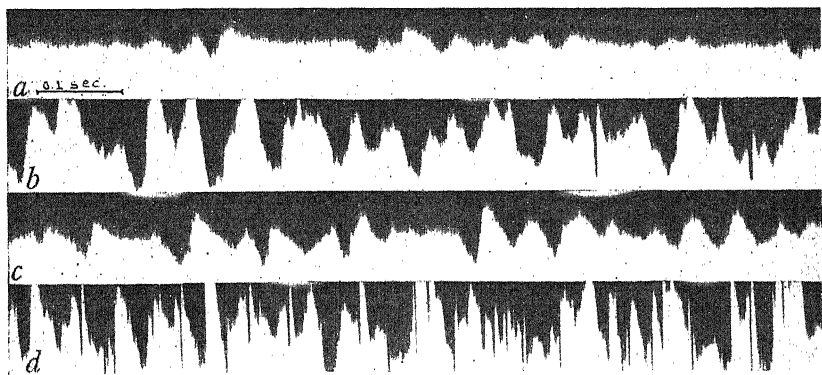
Although waves were clearly excited or augmented by the stimulating procedures, their relationship to the sensory activity is not entirely clear. Commonly the waves developed after a greater or lesser amount of sensory discharge. Yet exceptional experiments such as those of Text-figs. 2 and 4 yielded records of waves accompanied by few or no impulses. However, this does not mean that sensory impulses were not being aroused by the stimulation, but only that they were not carried by the particular ramus in use as lead. For in several instances, by shifting to another lead, sensory impulses were registered in numbers.

The question of the validity of the waves as evidence of nervous activity came early to the fore. The great effectiveness of acid in their production suggested an electrical artifact. However, the presence of



Text-fig. 3. Preparation of gastro-intestinal tract suspended in air, cannulae in oesophagus and rectum; electrodes 4 mm. apart on right ramus intercommunicans V to VI.

a, control, showing spontaneous discharge, and three fast impulses obtained by putting tension on the root of the mesentery. *b*, gastro-intestinal tract inflated with 25 p.c. HCl and immediately deflated (time consumed—2 sec.). Record taken 1–2 sec. after deflation, showing fully developed waves and a few very slow impulses. *c*, 2 p.c. novocain applied to root of mesentery; record follows at once.



Text-fig. 4. Preparation of gastro-intestinal tract suspended in bath of Ringer's, cannulae in oesophagus and rectum; electrodes 3 mm. apart on right ramus intercommunicans V to IV.

a, control, showing largest waves developed. *b*, gastro-intestinal tract filled with 25 p.c. HCl; record 30 sec. later, showing enormous waves. *c*, 5 min. later, waves smaller but still present. *d*, 1 p.c. nicotine applied to region of ganglia; record at once, showing wave and impulses discharge (compare with discharge of Plate I *d*, in which no waves are visible).

waves in control records and their undoubted production by mechanical stimulation almost eliminated this possibility. Furthermore, experiments carried out with elaborate precautions to shield the electrodes from mechanical or chemical disturbance exhibited waves as before.

The necessity of nervous conduction between the point of stimulation and the electrodes was established by two series of experiments. In one series the nervous continuity was interrupted at the height of a wave discharge by a cut across the root of the mesentery, the mechanical and electrical continuity being maintained either by apposition of the cut tissues or by a thread soaked in Ringer's. In every case the waves rapidly subsided. In a second series 1 p.c. novocain was used to block conduction. When applied to the site of stimulation at the height of a wave discharge, the discharge again rapidly subsided. When applied to the ganglia or lead itself, as in the experiment of Text-fig. 3, the effect was almost instantaneous.

That the waves were not nervous artifact produced in some obscure fashion as the result of the passage of sensory impulses over the lead was proven by experiments such as those of Text-figs. 2 and 4, in which waves were recorded almost free of impulse activity. To these may be added the numerous control records showing the same phenomenon. The control records also seemed proof that the waves were not the result of drying or other abnormal conditions in the nerve. Yet experiments were made further to control this possibility. Active but stable preparations left without irrigation for 5 or 6 min., the time of the longest experiments with acid did not develop waves in excess of the controls. Yet in no other experiment was irrigation dispensed with for more than a minute at most. Excessive irrigation with Ringer was also tried out, and found to be harmless.

A basis in nervous activity must then be conceded to the waves, yet their nature is far from clear. Occasional well-developed diphasic waves such as those of Text-fig. 2 might be interpreted as ascending sensory impulses, though of an order of conduction slower than any so far measured. The shortness of the rami did not allow of the experiments necessary for an accurate determination, but the rate must frequently have been less than 0.1 m. per sec. On the other hand, the variation in size and duration of the waves with intensity and duration of stimulation renders this interpretation untenable. For with an all-or-none concept of nerve activity, frequency is the only variable by means of which changing intensity of stimulation, or adaptation may be registered by a nerve fibre.

However, the waves might still partake of the nature of impulses if they were summations of many small action potentials. Increase in the number of impulses would then account for augmentation of the wave. Some of the records (Plates I and II) give the impression of such summation. Yet it is fairly certain that the impulses in these cases belonged to the slow sensory group and were merely superimposed on the waves. The typical wave (Text-figs. 1 and 2) was often remarkably smooth. If such waves represented summation, the individual potentials must either have been so well compounded that they eluded detection, or so small that their spikes were of the order of magnitude of the amplifier effect disturbing the base line. Waves of this smooth sort have been recorded by Adrian, Bronk and Phillips [1932] from the sympathetic nerves of mammals, and interpreted as synchronized discharges in efferent sympathetic fibres.

The presence of nerve cells under or close to the electrodes opens up the possibility that the waves represented cell, not fibre, activity. The waves in the sympathetic rami undoubtedly bear a superficial resemblance to waves recorded from preparations containing nerve cells, such as the water beetle's ganglia [Adrian, 1931 *a*, 1932 *b*] and the goldfish's brain [Adrian and Buytendijk, 1931], even though these were of very much longer duration. However, when the points of application of the electrodes were shifted within the very short range allowed by the rami, the waves were not materially altered. Furthermore, the clearly diphasic character of the shorter waves, those least affected by amplifier distortion, argues strongly for the conducted nature of these potentials.

On the other hand, the time elapsing before the waves appeared, their slow augmentation and, on cessation of stimulation, their slow subsidence, all argue for cellular or synaptic activity at some point in their course. To these may be added the similar subsidence of the waves after conduction from the area of stimulation had been interrupted by knife or novocain. Light may be shed on this behaviour by the presence of waves both in the spontaneous activity of irritable preparations, and in the discharges definitely provoked from ganglia by the application of a drop of 1 p.c. nicotine or a crystal of salt. Such waves were best seen as a discharge was developing or diminishing, or when it was slight, as in Text-fig. 4*d*. At the height of a massive discharge such as that of Plate II *d*, the waves, if present, were totally obscured. The waves of such ganglionic discharges persisted after a cut made distal to the ganglia, but were eliminated by a cut between ganglia and electrodes. Clearly, they had origin in the ganglia.

Unfortunately in the frog, nicotine is useful only as a sympathetic stimulant. Paralysis can be reliably obtained only with applications which seriously impair or block all nervous conduction. The attempt to establish the presence or absence of a synapse of sympathetic type in the wave activity, and to localize it, was, therefore, defeated. However, Pl. II shows an experiment in which nicotine was used to the point of quieting considerably the spontaneous discharge of this originally quite unstable preparation, yet only to the point of a moderate depression of the fast impulses. The waves obtained after the application of nicotine by a further local application of acid, seem reduced out of proportion to the general degree of depression.

The nature of the waves remains, then, ambiguous. If they represent sensory activity, not single impulses but summations of impulses must be involved—summations of impulses either so minute or so well synchronized that their discrete nature is obscured. A synchronizing mechanism on the afferent side has been demonstrated in the eel's eye by Adrian and Matthews [1928]. It is not beyond possibility that the cellular plexuses of the viscera or ganglia contain something analogous. On the other hand synchronized discharge is the usual form of activity in the efferent sympathetic system. The appearance and behaviour of the waves is compatible with the concept of efferent activity. Yet an interpretation of the waves as efferent impulses postulates either an axone or a synaptic reflex mechanism in the ganglia. Of knowledge of such mechanisms there is a singular dearth, though Langley and Anderson [1894] have described one in the inferior mesenteric ganglion of the cat. The problem is full of possibilities, but requires for its solution extensive degenerative sections of the separate components of the rami, with examination of the residual activity. These have not been done.

*The contributions of the separate rami to the sensory
innervation of the viscera.*

Frogs found, on opening the abdominal cavity, to have exceptionally long thin rami were used for this study. The animals were already pithed. The sympathetic rami of one or both sides were ligated at their point of connection with the spinal nerves, and cut central to the ligature. The viscera were left *in situ*, and the animal mounted, back down, on the experimental stage. In turn, the cord between the ganglia V and IV, and each of the rami communicantes from V to X, were led from, and their contributions to the sensory innervation of the viscera mapped out. The numerous small rami of lead XI could not be successfully dealt with.

Clear-cut results on both varieties of sensory impulse elicited from the isolated preparations were obtained only at lead VII, and above. The rami IX and X were at best very short, and almost continually the path of spontaneous discharge against which only impulses of the size and sharpness of the fast type could be depended on to stand out. The examination is, therefore, complete only for the fast variety of sensory activity.

Large fast impulses, of the type excited by touch or slight tension, were conducted from the viscera through the intercommunicating ramus V to IV, and through the communicating rami V, VI and VII, and occasionally VIII. Twice, however, the cord between V and IV was devoid of this activity on the right side, and once a VII ramus was blank. The rami IX and X never registered fast impulses, not even in preparations in which spontaneous activity had been quieted by nicotine. The distribution of each ramus over the mesentery and viscera was wide, and the overlap enormous. Thus the mesentery of the entire gastro-intestinal tract and of all its appendages was constantly supplied by ramus V. But the "fast fibres" coming down the trunk in the intercommunicating ramus from level IV or above had the same distribution as far as the rectum. And in many frogs, ramus VI again covered much the same territory. The fast fibres of rami VI and VII, and those of VIII, when present, made contributions to the innervation of the root of the mesentery, but were directed more particularly to the peritoneum covering the genito-urinary system and the blood vessels of the mid-dorsal line. In the supply of the gastro-intestinal tract, the contributions from the two sides were completely intermingled.

The fast sensory mechanism of the genito-urinary system was concentrated toward the midline, and oriented toward the root of the mesentery. In the root of the mesentery, the distribution of the separate rami was indecipherable. But over the kidneys and blood vessels a certain degree of segmental arrangement obtained and overlap was less. Here also the rami of one side had a wider distribution on that side than across the midline, though the area between the kidneys was always bilaterally innervated. The kidneys were well supplied by the fast mechanism, with some extension on to the mesentery of the testis, and in one instance on to the testis itself. But the enormous expanse of mesovarium and of the mesentery of the oviducts was explored in vain for sensory activity of this and, indeed, of any sort. Again, no fast impulses were registered from the bladder. But the failure to lead from the rami of level XI may be a factor in these negative results.

Two preparations having exceptionally long thin nerves and registering excessively large action potentials, allowed exploration of the distribution of individual "fast fibres" in the periphery. An estimate of the minimum number of fibres contributing to a discharge was often arrived at from the records by noting minute differences in size and duration of the action potentials. Thus in Text-fig. 2 *c* two fibres are clearly in action, the one developing a diphasic, the other a monophasic potential. Such differences were exaggerated by the loud-speaker. The note of the "fast fibre" was a clear sharp snap, whose pitch was determined by the form and duration of the potential, and its loudness by the size. If the potentials developed by a group of fibres were large, slight differences in pitch and intensity of the notes of the individual members could easily be discriminated. Thus the individual fibres could be identified whenever excited.

When the individual "fast fibre" content of the sympathetic rami was analysed in this manner, from one to perhaps half a dozen fibres seemed commonly to be in action in each ramus. More than half a dozen would be hard to keep track of, in any case. The distribution of the individual fibres, when more than one was active, was always less wide than that of the ramus, yet the area supplied by each was surprisingly large, and the overlap extensive. For illustration, the left intercommunicating ramus V to IV carried, in the more clear-cut of the two experiments being described, a minimum of six fast fibres, numbered from 1 to 6 in the order of diminishing action potentials. Number 1 was distributed only to the root of the mesentery, concentrating around the spleen—a very characteristic termination for the loudest, fastest fibre. Number 2 was distributed to the root of the mesentery and along the blood vessels supplying the caudal two-thirds of the small intestine and the rectum. Number 3 contributed to the root of the mesentery in passing, and went on to supply the cardiac portion of the stomach and the border of the pancreas in two very distinct divisions. Number 4 was distributed to the mesentery of the small intestine including the root, generously overlapping 2 and encroaching on 3. Number 5 began at the root of the mesentery and just touched the tips of both kidneys, and the peritoneum between, but extended laterally on to the mesentery of the left testis. Number 6, a fibre with a softer note, overlapped the distribution of 3, but extended further to the lines of mesenteric attachment along the liver. The pyloric mesentery discharged no fast impulses through this ramus. The other rami of this preparation seemed to contain fewer "fast fibres." The Vth ramus supplied the mesentery of the entire

gastro-intestinal tract from cardia to rectum either with one fibre or with fibres whose notes were indistinguishable. The ramus VI sent one fibre to the upper half of the left kidney and over the mesentery of the left testis. Either a branch of this fibre or another fibre of the same note went to the rectal mesentery. Two other fibres were distributed through the stomach mesentery, especially in the region of the pancreas. While a fourth, after supplying the root of the mesentery, ascended as high as the liver, to supply the region of the gall bladder. The VII ramus contained one very rapid fibre excitable from the middle third of the left kidney, from the mesentery of the left testis, and over the midline to the border of the right kidney. Rami VIII, IX and X registered no fast impulses. The content and distribution of fast fibres in the rami on the right side was similar to, but not identical with that just described for the left.

The fast impulse mechanism is, then, characterized by a very wide distribution of each ramus, and even of each nerve fibre. As a rule a given area is never supplied exclusively by one fibre, or even by one ramus, and the overlap of fibres of the same ramus, and of adjacent whole rami is enormous. The closer to the root of the mesentery was the area being explored the more strictly was this true. The region of the root itself is innervated not only by each fibre in its passage through to more distal regions, but receives in addition one or more fibres for itself alone, which were commonly the fastest fibres of the lot. Thus the impression gained initially by study of the minute distribution of this activity is confirmed. The system of rapidly conducting sensory nerve fibres and their endings composes a visceral proprioceptive mechanism, as it were, organized to register tension developed in the mesentery and adjacent peritoneum. Indeed, it may almost be considered a single enormous tension receptor for the mesenteric root.

Slow impulses. The rami VIII and above could be studied for their content of slow sensory impulses, but not the rami below this level. Below VI even, the rami were commonly so short and so thick that small slowly conducted action potentials might fail entirely to register. In the rami VI and above, however, and in favourable specimens of VII and VIII, slow impulses were observed, coming from the entire viscera. The slow impulses were found to have approximately the same peripheral distribution as the fast impulses of the same ramus, except that they were elicitable, as described, not only from the mesentery and other peritoneal ligaments, but also from the hollow viscera, and from the parenchyma of solid organs. That the slow impulses had, however, a

distribution independent of the fast, was shown clearly in two specimens in which the right intercommunicating ramus V to IV carried no fast impulse. For both these nerves developed clear-cut slow impulses when pressure was applied to the pylorus or small intestine, or the mesentery was roughly handled.

Nicotine was once successfully used to quiet spontaneous activity without seriously impairing all conduction. In this case clear-cut slow impulses were led from the X ramus during stimulation of the bladder by pinching, unaccompanied, however, by any of the fast type. Could proper conditions of recording be established, it seems likely that the slow impulse mechanism would prove to have quite as wide a distribution through the rami as it has through one ramus to the periphery. But until such experiments are performed, knowledge of the distribution of the slow sensory activity through the rami to the viscera must remain incomplete.

DISCUSSION.

The sensory impulses excited by stimulation of the frog's viscera present the same range of size and rate of conduction as do the impulses excited from the skin. Again, a more or less continuous series of action potentials can be divided into a fast and a slow group—the point of divergence being, however, less clearly defined in the sympathetic system than in the somatic. In this connection, Bishop and Heinbecker [1930] did not find the distinct separation of A, B and C waves in the conducted action potentials of visceral nerves which Erlanger and Gasser had found for the somatic. The significant difference between the two groups of sensory impulses lies, again, in their manner of excitation. From both sites, skin and viscera, the slow sensory impulse represents the response of the tissue to injurious stimulation, to excessive pressure, to burning. This response may be obtained when the fast mechanism has been destroyed, by scraping off the epithelium in the one case or by the action of weak acid in the other. From the reactions attendant on such stimulation in the decerebrate frog, this sensory activity may be correlated with the production in the intact animal of the sensation pain.

The mechanism of rapidly conducted sensory impulses, on the contrary, does not exhibit the obvious similarity in skin and viscera shown by the slow mechanism. Fast impulses from the skin represent activity of a tactile ending. From the viscera, they are best evoked by tension. Yet these two modes of excitation both depend on mechanical deforma-

tion, only developed in each case on a different scale. A more impressive difference between the rapidly conducted sensory impulses from skin and viscera appears in their distribution. Wherever examined, in skin or viscera, the slow sensory mechanism seems to be ubiquitous. The failure to demonstrate its presence in certain viscera was more probably a deficiency in the experimental procedure than in the mechanism. In the skin the fast impulse activity has the same wide distribution. But in the viscera this mechanism is rather strictly limited to the mesentery and adjacent portions of the peritoneum, with an organization along the blood vessels and directed at the mesenteric root.

The sensory innervation of the frog's viscera exhibits, then, in the slow impulse mechanism the universal primitive sensibility to noxious stimulation, to which has been attached the term protopathic. The contrasting epicritic activity, recognized in the rapidly conducted impulses from the frog's skin, is, however, wanting in the viscera, and even in the skin its localizing value is not great. The fast mechanism of the viscera represents, on the contrary, a specialized ending or group of endings of local rather than general significance, a mechanism more comparable with proprioceptive mechanisms, on the whole, than with the tactile sensibility of the skin.

SUMMARY.

The action potentials developed in frog's sympathetic nerves during stimulation of the viscera have been studied by means of amplification and photographic recording. These potentials form a series in which three dominating types may be discerned. These have been called fast impulses, slow impulses and waves.

The fast impulses represent activity of a mechanism responding rather to tension than to touch. They are elicitable from the mesentery and adjacent peritoneum, but not from the hollow viscera or from the parenchyma of organs.

The slow impulses represent activity of a mechanism responding to injurious stimulation, and of very nearly ubiquitous distribution. The reactions of decerebrate frogs to the stimuli evoking this activity are those considered indicative of pain.

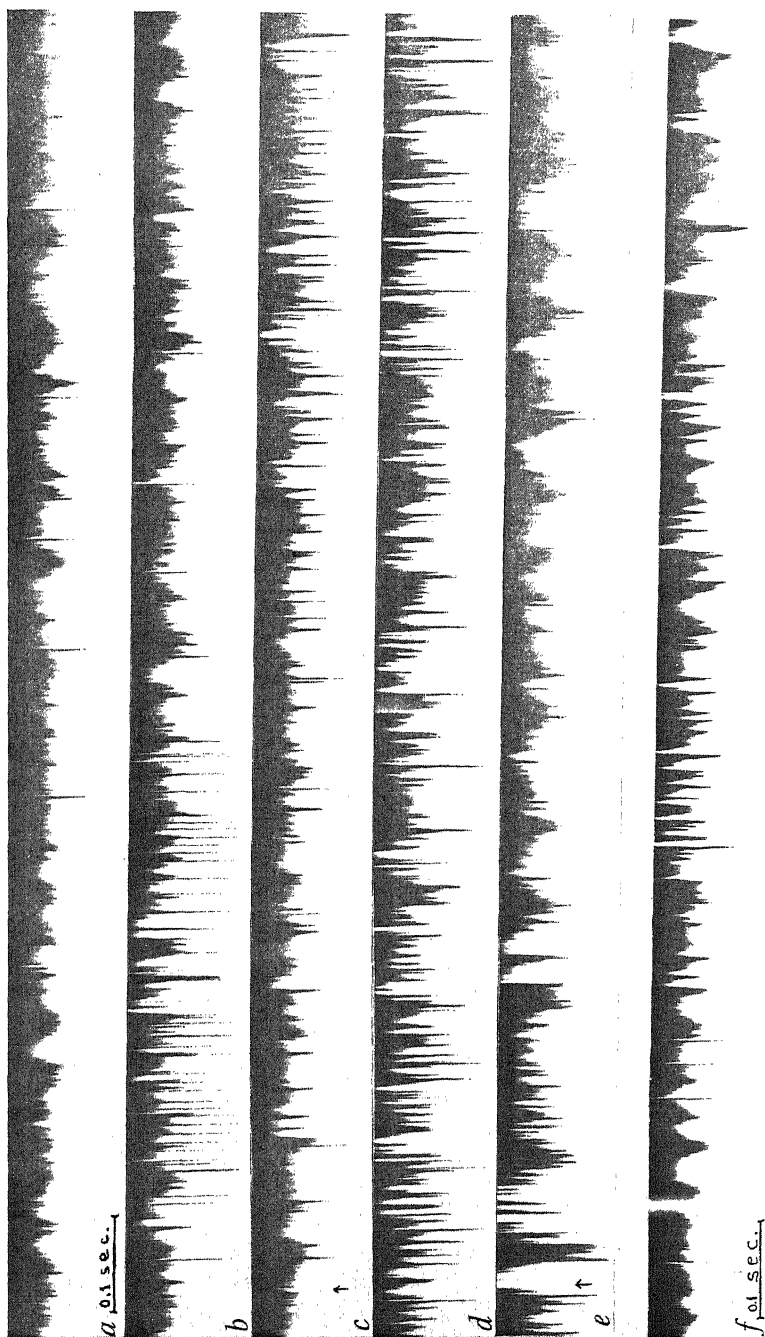
The waves represent an activity more difficult of analysis. Characteristically they are diphasic action potentials of duration less than 0.03 sec. They reach a maximum during sensory stimulation, but appear also in control records. They have been tentatively interpreted as synchronized discharges in efferent sympathetic fibres.

The contributions of individual sympathetic rami to the sensory innervation of the viscera have been investigated. In favourable material the peripheral distribution of single "fast fibres" has also been explored.

In this place it is a pleasure to record my indebtedness to Prof. E. D. Adrian, for inspiration and assistance at every stage of this work, from its inception to its imperfect conclusion.

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EXPLANATION OF PLATES I AND II.

PLATE I.

Types of potentials engendered by mechanical stimulation of the stomach and its mesentery. *a-e*. Preparation of male frog's entire viscera spread out on filter paper; electrodes 5 mm. apart on left ramus intercommunicans V to IV. Stimulation by blunt glass rod held in rack and pinion.

a, small fast impulses, from touching the mesentery in the pyloric angle of the stomach. *b*, fast and slow impulses, from touching the stomach mesentery near the root. A number of fast fibres are involved, producing potentials of different sizes and forms. *c*, moderately slow and very slow impulses, and small waves; pressure gradually applied to pyloric end of stomach. *d*, pressure maintained; impulse discharge continued; waves fully developed. *e*, pressure released; impulse discharge rapidly subsides and wave discharge, more slowly. *f*, preparation of stomach of female frog suspended in air by cannulae in the oesophagus and duodenum; electrodes 4 mm. apart on left ramus intercommunicans V to IV. Signal shadow marks beginning of rapid inflation of stomach with air. An initial discharge of three fast impulses due to stretching of the mesentery is followed by the discharge of slow impulse and by gradual augmentation of the waves present in the control record.

PLATE II.

Preparation of gastro-intestinal tract, spread out on filter paper; electrodes 3 mm. apart on right ramus intercommunicans V to IV.

a, control, showing spontaneous discharge of many ascending and one descending impulse, followed (arrow) by application of 25 p.c. HCl by a tiny cotton pledget to the middle of the loop of the small intestine. Discharge consists of fast and slow impulses and waves. *b*, 1 min. later, fast impulses have ceased, and waves are smaller. *c*, 5 min. later, control conditions almost restored, touching root of mesentery yields four fast impulses. *d*, control, followed by application (arrow) of two drops of 1 p.c. nicotine to the region of the V, VI and VII ganglia. *e*, 2 min. later, nicotine discharge over, spontaneous discharge greatly reduced (compare with *a* and *c*), fast impulses from touching root of mesentery also somewhat reduced (compare with *c*). *f*, control, showing one descending impulse, followed by application (arrow) of 25 p.c. HCl by cotton pledget to the pyloric angle of the stomach. Slow impulses only, followed by small waves; compare with waves of first record.

THE EFFICIENCY OF ISOLATED MUSCLE IN
RELATION TO THE DEGREE OF
AEROBIC ACTIVITY.

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IN the course of various investigations from this laboratory (*e.g.* see Hill, 1928 *b*) it has been found and repeatedly confirmed that the ratio of tension to initial heat production is an exceedingly constant quantity through a wide range of conditions, and further that the value of the delayed oxidative heat bears a constant relationship to the initial heat, equal to about 1.1. Actual experiments made with a view to comparing the efficiency of a muscle in long and short periods of activity have not hitherto been made, but in view of the above-mentioned observations any difference could not be large. Simonson and Hebestreit [1930] have recently published the results of some experiments on man, in the course of which the ratio of total work done to excess oxygen consumption was determined for a series of arm movements. A several-fold increase in efficiency was found in the series involving the larger amounts of exercise. Certain aspects of the methods employed by these workers seemed open to criticism, and, at the suggestion of Prof. A. V. Hill, have been re-examined by Dr Crowden, of the London School of Hygiene and Tropical Medicine, in a series of experiments on human subjects, while we have taken up the problem on isolated muscle.

Studies of efficiency in relation to duration of activity have a further interest at the present time on account of the recent findings regarding the chemical changes associated with muscular contraction. The discovery [Lundsgaard, 1930] that the breakdown of phosphagen yields energy available for work and the demonstration that under anaerobic conditions the magnitude of this breakdown in relation to the formation

of lactic acid varies with the number of twitches in a series [see Nachmansohn, 1928; Lundsgaard, 1931; and Meyerhof and Schulz, 1931] gave us grounds for expecting differences in the efficiency with which long and short periods of work are carried out. Evidence that such differences may occur has been obtained in the course of some recent experiments [Lundsgaard, 1931] in which determinations were made of the changes in phosphagen and lactic acid content of muscles subjected to various periods of activity under anaerobic conditions, from which the energy liberation could be calculated. On the basis of 100 calories as the heat of hydrolysis of phosphagen the calculations for the ratio of tension to heat show a definite decrease (lessened efficiency) as the duration of the series of twitches was increased¹. In some unpublished observations obtained by the myothermic method [Cattell and Parkinson] it has been observed that there is a gradual but rather small loss in efficiency in the course of a series of twitches in nitrogen. In the present experiments we have been concerned with the possible influence of the amount of activity on the efficiency of isolated frog muscle, as determined by measurements of the total tension and total heat production in an atmosphere of oxygen.

METHOD.

The observations were made on double sartorius preparations from English *R. temporaria* according to the general technique employed in this laboratory for a number of years [Hill, 1928 a]. Evaluation of the efficiency for various periods of activity was accomplished by taking the ratio of the total tension developed to the total heat production in a series of twitches in an atmosphere of oxygen. Stimulation at the desired frequency was secured by means of an induction coil in connection with a motor-driven contact breaker (Palmer) equipped with an arrangement for short-circuiting the make shocks. The muscles were mounted in the usual way in contact with the two faces of the thermopile (silver-constantan couples, shellac-bakelite insulation, and a glass cover) and connected with an isometric lever recording on a smoked drum. The muscle chamber was placed in a water bath at a temperature of 20.3° C. kept constant to 0.001 by means of a gas thermo-regulator. Before making observations the muscle was always soaked for about 2 hours in an oxygenated Ringer's fluid (pH 7.2; phosphate, 10 mg. *P* per 100 c.c.), a procedure which assures temperature equalization and favours the maintenance of normal irritability in the muscle.

¹ On the basis of higher estimates of the heat of hydrolysis of phosphagen the change would be less but still significant.

In many experiments the actual observations were made with the muscle immersed in paraffin oil, for the reason that it gives a greater stability [see Cattell and Hartree, 1932]. Later it was found desirable to soak the muscle in Ringer's fluid between observations, and for this purpose two reservoirs were arranged so that the fluid from either could be added through rubber connections to stop-cocks leading from the bottom of the muscle chamber. The reservoirs were kept immersed in the constant temperature bath at all times in order that the exchange of fluids could be made without seriously disturbing the galvanometer zero through temperature changes. Under these conditions observations could be continued within 5 minutes after changing the fluid. When empty (*i.e.* fluid in muscle chamber) the reservoir would float on the surface. When it was desired to remove the fluid from the chamber the reservoir was pushed to the bottom of the water bath, where it would remain on account of its increased weight as the fluid from the muscle chamber filled it.

RESULTS.

In any given preparation consistently lower values for the ratio of tension to total heat have been obtained for the longer periods of activity. We always alternated the long and short periods of activity in order that the possible influence of a change in the condition of the muscle might be suitably controlled. Evidence of a progressive loss in efficiency in a muscle maintained in an atmosphere of oxygen became apparent in the initial experiments. The results of some of these experiments will now be described in detail.

Exp. 1. Muscles maintained in an atmosphere of oxygen throughout the period of observation, after having previously been soaked for 2 hours in Ringer's fluid. The efficiency figure for the first short series of twitches in this and subsequent experiments was arbitrarily fixed at 100, and the succeeding series are expressed as a percentage of this value. The figures for efficiency are thus relative and have no absolute meaning. The numbers in parentheses following the efficiency percentage represent the time in minutes for complete recovery (*i.e.* return of galvanometer to zero). In this experiment the first value for the efficiency (100 p.c. [15 min.]) was obtained for 28 twitches over a period of 1 min. When recovery was complete a series of 127 twitches in 5 min. gave an efficiency figure of 81 p.c. (41 min.). A third series, this time consisting of 250 twitches in 10 min., gave a value of only 67 p.c. (87 min.). After these several periods of stimulation and after recovery was complete, a short

series, consisting of 26 twitches in 1 min., was again recorded, which now gave an efficiency figure of 71 p.c. (12 min.). The muscle was now soaked for $\frac{1}{2}$ hour in Ringer's solution, after which a repetition of the short series (28 twitches in 1 min.) showed an improvement in the ratio to 86 p.c. (13 min.).

Exp. 2. Conditions as in the last experiment. The values for the tension-heat ratio in a series of trials, in each case made up of about 20 twitches, were successively 100, 98, 103 and 104 p.c. The muscle was then subjected to a series of 140 stimuli in 7 min. (heat not recorded). After complete recovery the efficiency to another short series was 85 p.c., but the following one had risen to 94 p.c. Two subsequent short series, obtained after soaking the muscle in Ringer's fluid for $\frac{1}{2}$ hour, gave efficiency figures of 106 and 107 p.c. The recovery time in each of these periods of activity was between 15 and 17 min.

The foregoing examples indicate clearly a trend in the direction of a lessened efficiency with longer periods of stimulation. They also show that a muscle, maintained in oxygen alone, undergoes important changes in the course of an experiment, changes which may be largely prevented by frequent soaking of the muscle in Ringer's fluid. A similar decrease in the tension-heat ratio was observed in muscles maintained in oxygenated paraffin oil. For this reason in subsequent experiments it was made a regular practice to give the muscle frequent half-hour periods of immersion in Ringer's fluid, a procedure which was always followed both before and after the long stimulation series.

The third experiment, the data from which follow, illustrates another disturbing factor which was occasionally met with:

Exp. 3. The muscle was first soaked for the usual period in Ringer's fluid, after which oil was substituted and all the observations made in this medium. Initial series (efficiency 100 p.c.) consisted of 19 twitches, the recovery from which was complete in 15 min. Nitrogen was now passed through the oil instead of oxygen. Repetition of the short series now gave efficiency values of 146 and 140 p.c. in two trials, the increase being due to the absence of the oxidative recovery heat. After re-admission of oxygen a further short series gave a value of 98 p.c. (12 min.). The muscle was now stimulated 200 times in a period of 10 min., an amount of activity resulting in definite fatigue and giving a T/H value of 104 p.c. (51 min.). This was followed by a final short series which gave a value of 120 p.c. (10 min.).

The striking feature in this experiment is the rise in the T/H value in the last two series regardless of their duration. In such experiments, in the

absence of oil, the galvanometer fails to return to its original zero but comes to rest with a small positive deflection, indicating an increased vapour tension in the muscle [see Hill and Kupalov, 1930]. These facts clearly point to a condition of incomplete recovery, such as has long been known to occur in severely fatigued muscle, and thus invalidate the figures for efficiency. Three other preparations behaved in this manner, *i.e.* showed continuously rising values for the T/H ratio, and they have been omitted in the tabulation of the results. In each instance this effect occurred in muscles studied under paraffin oil, a fact which suggests that the oil or some impurity in it had an unfavourable influence upon the condition of the muscle. In this investigation the essential condition in order that valid conclusions may be drawn is that the efficiency values for the two short series, taken before and after the long series, must be in reasonable agreement.

TABLE I. The effect of the amount of activity on the efficiency of contraction.
See text for explanation.

Exp.	Short series	Long series		Short series
	p.c.	p.c.	Recovery time	p.c.
4	99 (19)	83 (112 in 4 min.)	48 min.	93 (21)
7	99 (20)	97 (98 " 2 ")	53 "	101 (20)
8	100 (18)	85 (78 " 1.3 ")	36 "	100 (17)
10	100 (17)	80 (70 " 1.1 ")	35 "	102 (17)
13	100 (18)	73 (140 " 2 ")	50 "	84 (20)
14	102 (19)	98 (54 " 1.3 ")	65 "	106 (17)
15	100 (22)	87 (105 " 4 ")	120 "	98 (18)
16	100 (17)	76 (77 " 1.3 ")	90 "	95 (17)
Average 100		84.9		97.4

With the exception of the experiments mentioned above and two others, discarded because of the introduction of a variation in technique which proved to be inadequate, all the results are summarized in Table I. All the experiments were carried out in an atmosphere of oxygen, except Nos. 7 and 8, in which the muscle was immersed in oil saturated with oxygen. In each experiment figures are given for the initial short series, the long series, and the final control short series. In every case the values are expressed as a percentage of the initial short series. The actual values shown in the table for the initial short series are not always exactly 100, for the reason that in most cases they represent the average of more than one observation. The figures in parentheses represent the number of twitches and these, in the case of the short series, were always distributed over a period of 1 min. In the case of the long series the time was variable and is indicated by the second figure within the parentheses. The time

for complete recovery for the short series of about 20 twitches was uniformly between 12 and 17 min. and therefore the individual values are not given in the table. In the case of the long series, where the total activity was variable, the recovery period showed correspondingly wide variations and is shown in the table in a separate column.

In this group of experiments it will be seen that in the prolonged periods of activity the T/H ratios were regularly less than in the shorter periods, the difference averaging 14 p.c. Good agreement was obtained between the short series of twitches taken before and after the long series, and we believe that the lower values of the T/H ratio, obtained in the latter case, represent a true lowering of the efficiency.

DISCUSSION.

In evaluating the results given above several possible sources of error must be considered. The only criterion of the completion of the recovery process is the return of the galvanometer to its initial position. With the longer periods of stimulation the recovery process is greatly prolonged so that any extraneous factor influencing the galvanometer would cause a relatively large error. That there was no chance disturbance causing a drift is shown by the fact that the galvanometer reading never continued past the original zero and the figure for the efficiency in the long series was uniformly less than in the short series.

The effect of activity on the osmotic pressure of muscle has been studied in detail by Hill and Kupalov [1930]. When the recovery processes are prevented, as, for example, in an atmosphere of nitrogen, the accumulated products of contraction increase the osmotic pressure and consequently moisture condenses on the muscle and results in a definite rise in temperature. This is a complicating factor in all determinations of the recovery heat, except those made under oil, because, until recovery is complete, the products of activity thus indirectly add heat to the muscle. Under ordinary circumstances this must be a negligible factor, but, following a prolonged period of activity, the recovery processes may continue at a low level for 40 min. or more. Even a rather small displacement of the galvanometer deflection over this period, due to an increased osmotic pressure, might conceivably represent an appreciable fraction of the heat recorded. This factor, however, could at most account for but a small part of the decreased efficiency observed for the longer series of twitches, and it is ruled out in those experiments in which the muscle was immersed in oil.

A second source of error, namely incomplete recovery, would result in low heat readings and affect the efficiency figure in the opposite direction, *i.e.* the value would be too high. This occurs when the muscle is fatigued and is illustrated above in the protocol of Exp. 3. The fact that in our experiments there was generally good agreement between the initial short series and the control taken after the long series is satisfactory evidence that the muscle was in good condition. The possibility remains, however, that the recovery processes may not have gone so far in the long series as in the short, and this suggestion is supported by the circumstance that frequently following the long series of twitches there persisted a small deflection of 2–5 mm. on the galvanometer scale. Under these circumstances we are inclined to believe that the average efficiency loss of almost 14 p.c. found in our experiments is rather less than the true value.

It is clear from Lundsgaard's experiments [1930] with muscles contracting without lactic acid production that under anaerobic conditions there is another source of energy—the breakdown of phosphagen—which is utilized for work. The work of Nachmansohn [1928] demonstrated that the breakdown of phosphagen is relatively greater in a short series of twitches than in a longer series. Lundsgaard [1931] and also Meyerhof and Schulz [1931] have shown that the lactic acid formation per unit of tension increases with the number of twitches in a series and at the same time the phosphagen breakdown becomes less. It thus appears that the energy for a short period of anaerobic work is derived largely from phosphagen, while in more prolonged activity the formation of lactic acid becomes of increasing importance. On the basis of Lundsgaard's hypothesis, that the energy arising from the formation of lactic acid is used for the resynthesis of phosphagen and only through this cycle becomes available for doing work, a decrease in the efficiency with the prolongation of anaerobic activity is to be expected unless one can assume that under these conditions the process of phosphagen restoration is carried on without loss of energy. The calculation of Burk [1929], according to which the free energy of the breakdown of glycogen to lactic acid is considerably higher than the heat formation, makes a high efficiency for the anaerobic resynthesis of phosphagen at any rate possible. The fact [Fischer, 1930; Cattell, Feng, Hartree, Hill and Parkinson, 1931; Hartree, 1931] that in muscles contracting without the formation of lactic acid (due to the action of monoiodo-acetate) the ratio of the initial heat per unit length to the tension lies within normal limits seems to indicate that the loss of energy cannot be large. However, the

experiments of Cattell and Hartree [1932], showing the production of delayed heat following a series of twitches in nitrogen amounting to about 20 p.c. of the value of the initial heat, such as has long been known to occur in the case of a short tetanus, point to a loss in efficiency, since the delayed anaerobic heat is most probably due to an energy loss incurred during the resynthesis of phosphagen. Further, it was shown that the delayed anaerobic heat falls to an extremely low value (about 5 p.c. of the initial heat) when the series is reduced to but a few twitches, and this very possibly is significant of a difference in efficiency of the kind observed in the present experiments.

Because of the relatively high frequency of stimulation employed in our experiments, the muscle, although in an environment of oxygen, must have been working to a certain extent anaerobically. It may, therefore, be assumed that the fraction of the initial heat originating from the breakdown of phosphagen was greater in the short series. How great is the difference in the ratio of phosphagen to glycogen breakdown between the long and short series it is not possible to say, but, since a certain amount of recovery must have occurred during the long series, it is not likely that the difference was large.

From what has been said above it seems unlikely that the rather large differences in efficiency observed can be accounted for wholly on the basis of the chemical changes occurring in the initial phase of contraction. In addition it is necessary to consider possible differences between the long and short series in the recovery phase. Just as the initial heat originates from at least two different processes (phosphagen breakdown and lactic acid formation), two different restorative processes take place during aerobic recovery (the resynthesis of phosphagen and restoration of glycogen). It is possible, perhaps probable, that the efficiency of these separate endothermic processes is not the same. If this is the case the efficiency of the muscle contracting in oxygen would be affected by differences in the ratio of the initial heat from phosphagen to the initial heat from glycogen. In our experiments such a change of efficiency was observed, lower values appearing under circumstances in which the above-mentioned ratio must have been the smallest (long series). This would lead to the conclusion that the efficiency of aerobic phosphagen resynthesis is higher than that involving the restoration of glycogen. Strong support for this conception comes from the recent experiments of Cattell and Shorr [1932] who, working on mammalian muscles, found that the ratio of the recovery heat to the initial heat was approximately twice as great in a one-second tetanus as that in a series of three to five

twitches, denoting a corresponding difference in the efficiency of the recovery processes. The result of the only attempt directly to measure the oxygen consumed during aerobic phosphagen resynthesis [Meyerhof and Nachmansohn, 1930] is in favour of the assumption that this process is carried on with a high degree of efficiency.

There is a further possibility, *i.e.* a change in Meyerhof's oxidative quotient, which must be considered in this connection. A small increase in the proportion of lactic acid (or its equivalent of other material) oxidized during the recovery period would account for a lower efficiency figure. Such a difference might well be associated with the extra accumulation of lactic acid occurring as the result of the greater degree of activity.

Our results obtained through observations on isolated frog muscle appear to be in complete disagreement with those of Simonson and Hebestreit [1930] who, working on human subjects, found a several-fold increase in efficiency with increasing duration of work. This result is at variance with a large body of data which has accumulated during recent years from this and other laboratories and is almost certainly incorrect. In carefully controlled experiments Dr Crowden has recently made a study of the efficiency for short and long bouts of exercise on the stationary bicycle and has not been able to detect any changes in efficiency with different durations of exercise.

In connection, however, with the results of Simonson and Hebestreit we should like to point out the difficulty in making a valid comparison with data obtained on isolated muscle. It is obviously impossible to compare the work performed by human muscles having a normal circulation with the twitches of an isolated frog's muscle supplied with oxygen through diffusion from the surface, or to determine corresponding degrees of anaerobiosis. The possibility exists that the short series in our experiments should be compared with the long series of Simonson and Hebestreit and *vice versa*. In the intact muscle the oxygen concentration is undoubtedly relatively low at the beginning of an exercise period when the oxygen demands are high and the compensatory changes in the circulation have not yet taken place. During this period an oxygen debt is set up. Later, during the "steady state," the oxygen debt is not further increased but may even decrease, and thus the work done by the muscle is purely aerobic. The possibility exists that during this period the initial heat is derived exclusively from a breakdown of phosphagen and that this is restored by means of oxidation energy without the intermediate formation of lactic acid. In that case the ratio of the initial heat

from phosphagen to the initial heat from glycogen, and hence the efficiency, should increase, to a certain extent at least, with increasing duration of work. The conditions would then be exactly opposite to those obtaining in isolated muscle and discussed above. In view of the extremely big changes in efficiency reported by Simonson and Hebestreit and the inability of Crowden to confirm them, it seems hardly probable that such considerations can reconcile the two sets of observations. We have thought it worth while, nevertheless, to enter into a discussion of them in order to point out the difficulties of making a comparison of results obtained on isolated frog muscle with those from man and to emphasize our view that the ratio of the initial heat from glycogen to the initial heat from phosphagen—which is likely to change with the duration of work—may markedly affect the efficiency.

SUMMARY.

1. In the isolated sartorius muscle of the frog, stimulated in oxygen, the efficiency of isometric contraction was found to be higher in a short series of twitches than in a long series.

2. If there is a difference in the economy of the processes by which glycogen and phosphagen are restored in the presence of oxygen, the ratio of the initial heat derived from phosphagen to the initial heat from glycogen must influence the efficiency of doing work. The results are in agreement with the assumption that under aerobic conditions the re-synthesis of phosphagen is carried on with a higher efficiency than the restoration of glycogen.

3. The observations of Simonson and Hebestreit on man are discussed in relation to our findings.

We wish to extend our thanks to Prof. A. V. Hill for his many courtesies to us during our stay in his laboratory.

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THE ACTION OF ETHER ON THE
SYMPATHETIC SYSTEM.

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THE usual pharmacological teaching concerning the action of ether is that, like other anæsthetics, it produces a descending paralysis of the central nervous system. Ether is said to be preferable to chloroform because it has very little action on the heart, and the chief danger attending its use is held to be that of post-operative pneumonia, which occurs because of the irritation of the bronchial mucous membrane.

The effect of ether on the blood-pressure is well known to laboratory workers. In a cat under ether, with the vagi cut, the blood-pressure is usually very high; but an increase in the depth of anæsthesia causes a fall.

The cardio-vascular effect of ether has been analysed by Cattell [1923] in a valuable paper which includes a full review of earlier work. Cattell showed that, in cats, the sudden inhalation of a high concentration of ether vapour often caused a drop in the blood-pressure followed by recovery to the previous, or to a still higher, level. In animals suffering from shock, however, there was no recovery. From the very clear results obtained in experiments in which cardiometer records were taken, Cattell concluded that the drop in blood-pressure was due to weakening of the heart and diminished output, while the recovery was due to increased vaso-constriction caused either by a reflex initiated by the fall of pressure, or by a direct stimulant action of ether on the medullary centre. His evidence for the occurrence of vaso-constriction was obtained from (a) plethysmograph experiments in which he observed that inhalation of ether caused diminution of limb volume, which was not always attended by fall in blood-pressure, and which did not occur if the nerves were severed; (b) perfusion rate experiments in which he observed that the time taken for a given volume of saline to flow through a limb, by way of a branch of the main artery during temporary arrest of the blood flow, was increased during administration of ether; (c) observations

that intra-arterial injections of saline, saturated with ether, caused a rise of blood-pressure, whereas intravenous injections caused a fall.

Macdonald and Schlapp [1926] have published a short note pointing out that the action of small doses of adrenaline on the blood-pressure is dependent on the presence or absence of an anæsthetic. In their note no tracings were published, but Dr Macdonald has kindly shown his tracings to us. Macdonald and Schlapp found that in a decerebrate cat, still under the influence of ether, doses of adrenaline from 2γ to 10γ caused a fall of blood-pressure; when the ether was entirely removed these doses were mainly pressor; when ether was again administered, the doses were again depressor, despite the fact that the blood-pressure was lower than when ether was absent. It may be noted that urethane (but not chloralose) was found to behave like ether; under chloralose small doses of adrenaline had much less depressor action.

G. H. Miller [1926] has studied the action of ether and chloroform on the movements of the gastro-intestinal tract. He speaks of "the prevailing impression that general anæsthesia has little effect" on these movements and attributes this impression to the results of Cannon and Murphy [1906]. These workers were not concerned with the effect of the anæsthetic itself, but were demonstrating that handling or trauma of the intestines during ether anæsthesia causes much more delay in the recovery of normal movement than does anæsthesia alone. Miller showed, by experiments on dogs with gastric and intestinal fistulæ, that "on beginning a very slow induction of anæsthesia with ether, there occurs almost immediately in stomach, small intestine and colon a diminution in size of contractions and an associated slight loss of tone." Later in his paper he stated that the relaxation of the gastro-intestinal tract was synchronous with the onset of third stage anæsthesia.

Shafer, Underwood and Gaynor [1930] showed that the effect of vagus stimulation on the heart rate was much less in dogs anæsthetized with ether than in the same animals after decerebration; they confirmed the finding of Lieb and Mulinos [1929] that, in certain stages of amyotal anæsthesia, stimulation of the vagus had no inhibitory effect on the heart.

It is surprising that in all the papers which have been published on the action of ether, even in Cattell's otherwise complete review, there is no reference to the demonstration of Elliott [1912] that during ether or chloroform anæsthesia the store of adrenaline in the suprarenal glands is steadily diminished by impulses which pass along the splanchnic nerves. It is evident that, since ether depletes the store of adrenaline,

there is probably a continual discharge of adrenaline into the blood stream, and that some effects of ether, such as the vaso-constriction observed by Cattell [1923] or the intestinal paralysis described by Miller [1926], may be caused, in part at least, by this discharge. Moreover since the effect of ether on the suprarenals is due to impulses passing along the splanchnic nerves, it is reasonable to look for evidence that ether causes a discharge of sympathetic impulses to other organs.

One of us [Burn, 1922] observed that when ether was administered to kittens in which the stellate ganglion had previously been extirpated on one side, there was sweating on the normally innervated fore-paw, but none on the paw in which the sympathetic fibres had degenerated. This sweating was clearly due to central influences, but it was not certain whether the ether caused the sweating as a primary or as a secondary effect. Bourne and Burn [1927] observed that, when ether was administered to patients in the first stage of labour, there was an immediate cessation of the contractions of the uterus; a similar effect was obtained when adrenaline was injected into a vein. That adrenaline caused inhibition was taken to indicate that sympathetic stimulation would also cause inhibition; hence the effect of ether might be ascribed to a discharge of impulses along the sympathetic nerves, or alternatively to the liberation of adrenaline from the suprarenal glands.

The observations of Elliott, the observations on sweating and those on intra-uterine pressure, suggest the possibility that ether and other anæsthetics like chloroform and urethane, cause a continuous discharge of impulses, not merely to the suprarenal glands, but in other sympathetic fibres. We have investigated this possibility.

EXPERIMENTAL RESULTS.

1. *General procedure.*

Observations have been made on cats which were anæsthetized with ether, and in which the suprarenals were completely excluded from the circulation by ligaturing all the vessels. Either each gland was completely excised, or, after the ligatures were tied, it was cut open to ensure that the circulation through it had ceased. Some of the cats so prepared were then decerebrated by removing the brain through a trephine hole in the skull, the brain stem being divided in the plane of the tentorium. In other cats the spinal cord was divided at the second cervical vertebra and the brain was destroyed by a probe passed into the foramen magnum. In other cats, in which the spinal cord was cut, the whole spinal cord was

destroyed as well as the brain by passing an umbrella wire down the spinal canal. We are indebted to Dr E. G. T. Liddell for showing us the details of decerebration.

2. *Observations on the spleen.*

Changes in the spleen volume were recorded by enclosing the spleen in a plethysmograph. An incision was made parallel to the spinal column, through the muscles of the flank, and the spleen was gently drawn outside the body of the cat. The plethysmograph was a wide boiling tube, on the side of which was blown a circular hole with a flanged edge, so that a thin rubber membrane could be stretched over the hole. A longitudinal incision was made in the membrane, and the spleen was tucked inside the boiling tube through this incision. When the boiling tube was clamped in position by the side of the cat, and the open end was fitted with a stopper bearing a tube connected to a piston recorder, it was found that the changes in spleen volume were recorded with ease.

Before the observations were made, cats were left for about 2 hours after the decerebration or after the cutting of the spinal cord. The best results were obtained 3 or 4 hours after the decerebration. Care was taken to keep the animals warm. The vagi were cut, the animals were ventilated with warm air (although the natural respiration was maintained); occasionally if the blood-pressure was very low, a dilution of pituitary (posterior lobe) extract in saline (0.2 unit per c.c.) was infused into the femoral vein at a constant rate, about 1 c.c. per minute.

A wide-tubed wash bottle containing ether was introduced on the course of the tube carrying the air from the pump to the trachea. When a screw clamp on a short-circuiting tube was unscrewed, the air from the pump reached the cat without passing through the ether; when the screw clamp was closed, all the air passed through the ether. Ether was usually administered by giving the screw a half turn, so that a portion of the air passed through ether.

Using the decerebrate preparation, it was found that the administration of ether in the period 3 or 4 hours after decerebration, when the blood-pressure was not unduly high, was regularly attended by a prompt fall in the volume of the spleen, and that the discontinuance of the ether was followed by a rise in this volume. The change is illustrated in the left-hand portion of Fig. 1, in which it will be observed that there is little accompanying fall of blood-pressure; it was evident from this and several similar experiments that the change in spleen volume was not secondary to a change in blood-pressure. In some of the experiments the effect was

observed about 1 hour after decerebration; exceptionally it was not observed until 3 or 4 hours had elapsed; when the blood-pressure was high it was less easy to observe the response.

This result supports the view that the administration of ether results in a discharge of sympathetic impulses not merely to the suprarenal

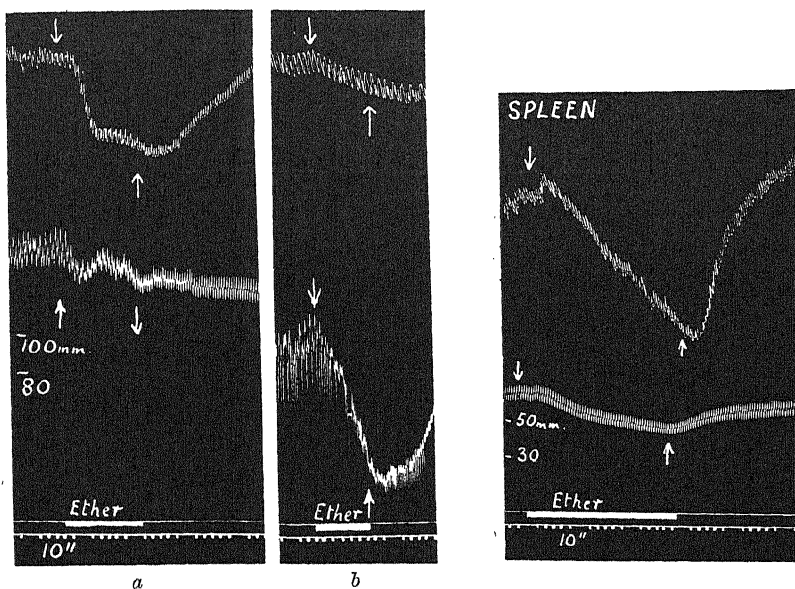


Fig. 1.

Fig. 2.

Fig. 1. Record of spleen volume and blood-pressure in decerebrate cat from which the suprarenals were removed. The vagi were cut. On the left the administration of ether in the air pumped into the lungs produced a contraction of the spleen, but little change of blood-pressure. Nicotine was then administered until the pressor action disappeared. On the right the administration of ether did not affect the spleen, but produced a steep fall of blood-pressure.

Fig. 2. Record similar to that of Fig. 1 in a spinal cat. Note the diminution of spleen volume during the administration of ether (between arrows) and the prompt recovery when ether was discontinued.

glands. We conceived that the site of action of the ether was the medulla, and expected to find that, when observations were made on the spinal cat in which all nervous tissue above the second cervical vertebra was destroyed, a motor effect on the spleen would no longer be seen. In this we were wrong, as is shown in Fig. 2. The experiment was performed, as before, after exclusion of the suprarenals from the circulation, and

administration of ether was attended by a fall in the spleen volume, which rose again as soon as the ether was discontinued.

If the impulses which lead to the fall in the spleen volume do, in fact, pass along the sympathetic pathway, their passage should be prevented by the administration of sufficient nicotine to paralyse the ganglia. We have observed that this is so, as the right-hand portion of Fig. 1 illustrates. In normal cats the amount of nicotine (acid tartrate) required to produce full paralysis is about 15 mg. per kg.; after removal or exclusion of the suprarenals the amount is only 2-3 mg. per kg. After doses of nicotine there is often cardiac irregularity, as seen in Fig. 1, but the same absence

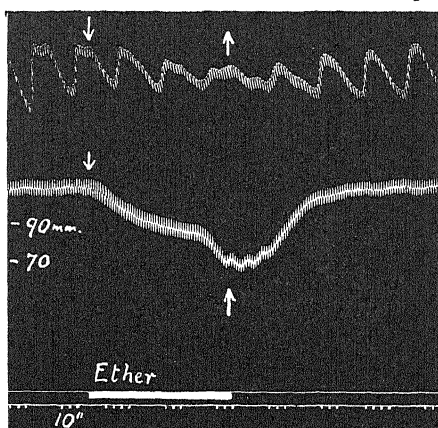


Fig. 3. Record shows that the administration of ether in the fully pithed cat does not cause contraction of the spleen, but diminishes the rhythmical changes in the spleen volume which are seen so strikingly in the fully pithed cat. Ether administered between the arrows.

of change in the spleen volume on administration of ether after nicotine was observed in other cats in which the cardiac irregularity was not evident. The much greater fall of blood-pressure produced by ether after nicotine paralysis will be considered later.

Final evidence that the site of action of ether in producing contraction of the spleen is the central nervous system was furnished by experiments on cats, in which the spinal cord was destroyed as well as the brain; in all of these there was no contraction of the spleen when ether was given. It was observed that, when the spinal cord was destroyed, the spleen volume displayed rhythmic contractions. The administration of ether diminished these contractions, but never produced a fall in spleen volume; an illustration of the effect is given in Fig. 3.

3. *Observations on the intestines.*

We have recorded the movements of the intestines in two ways: firstly by recording the variations in the distance between two points on a piece of small intestine, a silk through the wall fixing the intestine at one point to a vertically supported glass rod, and a hook through the wall at the other point being connected to a writing lever. When ether was administered to a decerebrate cat from which the suprarenals had been removed, lengthening, that is to say relaxation, of the intestine wall was observed, and, so soon as the ether was discontinued, a prompt and complete recovery took place.

Inhibition of intestinal movements was also observed by inserting a bag in the lumen of the intestine. The bag was prepared by tying a portion of a rubber finger-stall on the end of a gum-elastic catheter; the bag and catheter were then filled with water. The portion of intestine selected was picked up and incised transversely so as to admit the bag; the incision was sewn together with silk. The end of the catheter was attached by a rubber connection to a glass tube supported vertically, and this was connected to a piston recorder. Fig. 4 shows a record of the effect of ether on the intestinal movements when pituitary (posterior lobe) extract, 0.2 units per c.c., was infused at a constant rate into the femoral vein. The movements were arrested by ether, and the tone was inhibited, to be re-established when the ether was stopped.

The same arrest of intestinal movements was observed in the spinal animal, and the resumption of tone and rhythm when ether was removed was just as prompt as in the decerebrate animal. In the fully pithed animal, in which the spinal cord was destroyed, the rhythmic movements were much smaller and there was less tone in the intestines even when pituitary (posterior lobe) extract was infused into a vein. Consequently it was more difficult to observe the effect of ether. It was found, however, that the administration of ether lessened tone and rhythm in most experiments, though not in all, but the effect was gradual and not abrupt as in the decerebrate or spinal preparation (see Fig. 5). Furthermore, when the ether was removed, the movements did not start again; this was the most striking difference from the effect observed when the spinal cord was intact. Evidently ether exerts a depressant action, either by stimulating the sympathetic ganglia, or by paralysing the cells of Auerbach's plexus, or by acting on the muscle itself; this depressant action is, however, not the cause of the inhibition observed when the spinal cord is intact, for this inhibition disappears as soon as the ether is removed.

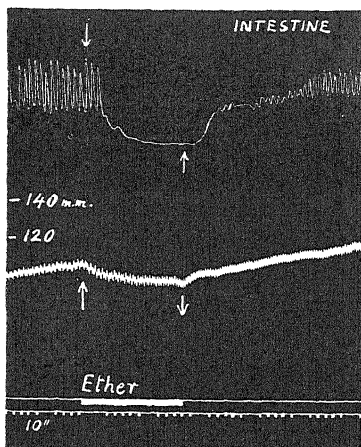


Fig. 4.

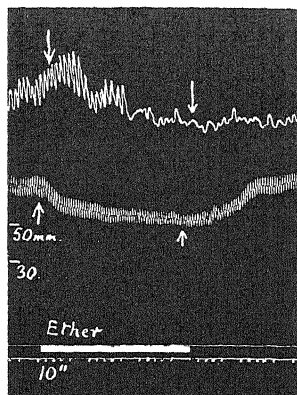


Fig. 5.

Fig. 4. Effect of administration of ether on the pressure inside a balloon inserted in the small intestine. The cat was decerebrated, the suprarenals were removed, and an infusion of pituitary extract was made into the femoral vein at a constant rate. Note the prompt inhibition when ether was given, and the recovery when it was cut off.

Fig. 5. The effect of ether on the intestinal movements recorded in the same way as Fig. 4, in a fully pithed cat, into a vein of which pituitary extract was infused. Note the absence of a prompt inhibition when ether was given, and the failure of the tone to re-establish itself when ether was discontinued. The failure was lasting in all experiments after destruction of the spinal cord.

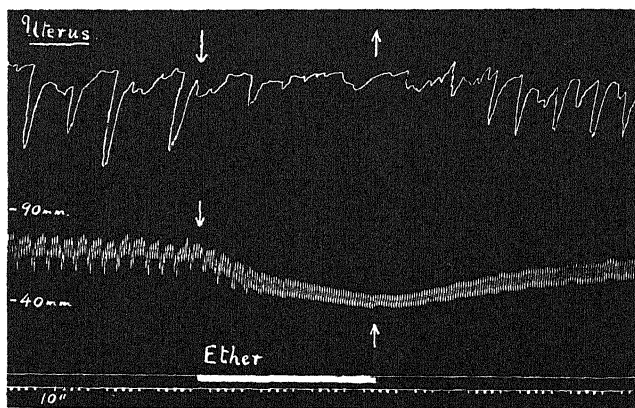


Fig. 6. Effect of ether in inhibiting the contractions of the uterus of a virgin cat after decerebration. The suprarenals were removed. Pituitary extract was infused into a vein. The movements restarted soon after the ether was discontinued.

4. *Observations on the uterus.*

We have made observations on the virgin cat uterus, recording its movements *in situ*, by fixing one end of one horn to an upright glass rod with a silk, and by inserting in the other end of the horn a hook attached to a writing lever. To enable the record to be more easily made, the stomach and intestines were excised, and to stimulate regular contractions in the uterus, pituitary (posterior lobe) extract, of strength 0.2 unit per c.c., was infused into a vein at a constant rate. It was observed that when ether was administered, the movements were at once arrested, and that when the administration stopped, the movements soon restarted (see Fig. 6).

5. *Observations on the heart rate.*

When ether is administered to a decerebrate cat in which the vagi have been cut and from which the suprarenals have been excised, the heart rate is quickened. The following figures exemplify this.

Heart rate before ether administered, 143 per minute;

„ „ during ether, 158 per minute;

„ „ 2 minutes after ether stopped, 151 per minute.

6. *Observations on the pupil and the bronchioles.*

Some inconclusive observations made on the pupils and on the bronchioles may be mentioned. In a cat under urethane, in which the suprarenal glands were excised and in which the right cervical sympathetic chain had been cut, it was observed that administration of ether caused dilatation of the pupil first in the left eye, but that dilatation was later seen in the right eye; sometimes, however, the dilatation of the two eyes was simultaneous. Observations were also made on the air entry into the trachea in decerebrate cats breathing spontaneously; a side tube in the tracheal cannula was connected to a piston recorder; it was observed in some experiments that, when ether was administered, there was an initial increase in the air entry which we took to indicate dilatation of the bronchioles.

7. *Observations with chloroform and urethane.*

We have observed that chloroform acts in the same way as ether in the decerebrate and spinal cat in causing contraction of the spleen, and that it fails to cause this contraction in the fully pithed animal. We also studied the effect of injecting a solution of urethane; we found that this substance caused an increase in the rhythmic contractions of the spleen, but no certain decrease in volume.

8. *Cardio-vascular action of ether.*

Effect in the heart-lung preparation. In view of the work of Cattell [1923] we have made no general study of the action of ether on the heart and vessels. Cattell showed that the fall of blood-pressure which ether causes is due to a weakening of the action of the heart. His evidence on this point has not gained notice in text-books on pharmacology, for, in these, emphasis is usually laid on the difference between chloroform and ether. Cattell's observations were made by means of cardiometer records in the intact animal; to put the matter beyond doubt, we have made observations on the heart-lung preparation of the dog, recording the changes in the heart volume. Our observations show that earlier statements that chloroform weakens the heart more than ether are correct. The mixture of oxygen with 5 p.c. carbon dioxide with which the lungs were ventilated was blown through a layer of ether of given depth for exactly 10 sec.; the effect on the heart was observed. The effect was again observed, substituting chloroform for ether, and then again with ether. The dilatation of heart volume was much greater with chloroform. Nevertheless, as Fig. 7 shows, the administration of ether for quite short periods such as 80 sec. results in an immediate weakening of the power of the heart muscle; the volume increases and the blood-pressure previously maintained falls. The volume increase persists for a considerable time after the administration of ether is stopped. There is no change in heart rate.

Effect on the blood-pressure. We have repeatedly observed that, when ether is administered to a decerebrate cat (from which the suprarenals have been removed), there is an initial rise of blood-pressure which does not last very long and which is followed by a return to the previous level or by a fall. This must be due to vaso-constriction, and confirms the findings of Cattell.

Additional evidence in favour of the occurrence of vaso-constriction is given by the effect of ether on the blood-pressure after full doses of

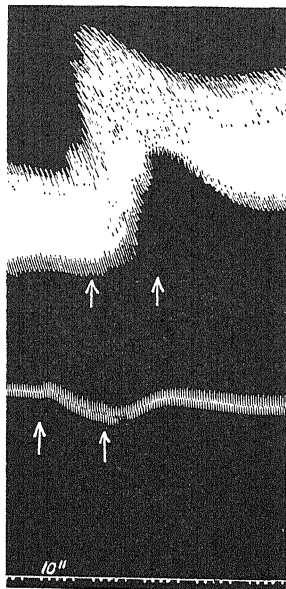


Fig. 7. Effect of moderate amount of ether for 80 sec. on the heart volume of a dog. (Heart-lung preparation.)

nicotine, when a profound fall is seen (see Fig. 1 *b*). Before nicotine was given, ether had no effect on the blood-pressure; after nicotine, the fall took place. We interpret the fall as due to the lessened output from the heart; no fall was observed before nicotine because simultaneous vaso-constriction neutralized the effect of the lessened output on the pressure. The effect of a lessened output is cumulative in the absence of simultaneous vaso-constriction because of the decline in the coronary flow.

Action on the perfused vessels. We have further observed the effect of ether on the vessels themselves. We have investigated the effect in the hind limb of the dog perfused with blood by means of the Dale-Schuster

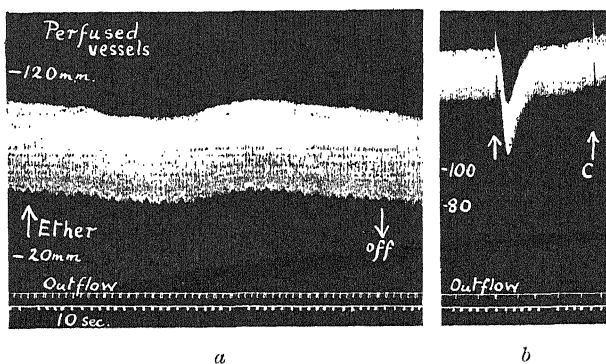


Fig. 8. Effect of ether on the vessels of the dog's hind limb perfused with defibrinated blood. On the left is shown the effect of ether vapour added to the oxygen used for ventilating the lungs. Plenty of ether entered the blood as the blood in the venous reservoir smelt strongly. There was little effect on the tone. On the right is shown the effect of an intra-arterial injection of 0.3 c.c. saline saturated with ether. 0.3 saline alone was given at *C*.

[1928] pump, the blood, after passing through the leg, being pumped through the lungs. Ether was administered in two ways; it was introduced through the lungs by blowing the ventilating air through ether and also by the injection of a saturated solution of ether in saline into the artery cannula. The effects are illustrated in Fig. 8 *a, b*. When the ether was introduced into the blood through the lungs, it was uncertain at what point the ether first reached the vessels of the leg; no abrupt change in vascular tone was observed. Fig. 8 *a* shows a slight fall in tone followed by a slight rise. Since the blood issuing from the leg veins smelt strongly of ether, it was evident that ether reaching the blood through the lungs has little effect. Fig. 8 *b* shows that when a saturated solution is injected there is a transient loss of tone.

DISCUSSION.

It is curious that Elliott's observations in 1912 that ether, chloroform and urethane, by means of sympathetic impulses, liberate adrenaline from the suprarenal glands, have not previously been generalized. We have observed that in decerebrate or spinal animals, deprived of the suprarenal glands, ether causes contraction of the spleen, inhibition of the intestine and uterus, and rise in the heart rate. The effect on the spleen is abolished by nicotine. In the fully pithed animal ether has no effect on the spleen, and the inhibition of the intestine is gradual in onset, persisting after removal of the ether. In the heart-lung preparation ether has no effect on heart rate. The evidence supports the view that ether acts on the central nervous system, so that all sympathetic nerves are stimulated; we regard our observations merely as an extension of the original observation of Elliott on the effect on the suprarenals.

It was a surprise to us to find that the effect of ether on the spleen and the intestine was seen in the spinal animal; it is evident that the stimulation is not confined to centres within the brain, but can act also on points within the spinal cord.

In view of Cattell's work [1923] we have made no special study of the effect on the vascular system, but we can add evidence confirming his conclusions. He found that the sudden administration of a high concentration of ether led to a fall in blood-pressure, followed by recovery; he attributed the fall to a weakening of the heart and the subsequent rise to a compensating increase in arterial tone. We have found that in the heart-lung preparation ether causes an immediate increase in cardiac volume, which lasts for a long time after the administration of ether ceases. This observation was very surprising in the light of the current pharmacological teaching that ether has little effect on the heart. Few opinions have been expressed on the effect of ether on the circulation, and it is difficult to know to what most observers attribute the fall of blood-pressure which occurs when ether anæsthesia is deepened. Clark [1932], in the fourth edition of *Applied Pharmacology*, says that "Ether has a less depressant action upon the medullary centres than has chloroform, and produces a less marked fall of blood pressure." We do not know what is the evidence for this statement of a depressant action, but we suspect it to be an inference from the common impression that the heart is little affected by ether. Cattell's evidence is that ether constricts the vessels by impulses proceeding from the central nervous system; this we can confirm from the observation that when central impulses are cut

out by nicotine, ether produces a much greater fall of pressure than before.

Since ether has no effect on the vessels themselves, the picture of the vascular system during anæsthesia is that of a heart seriously weakened by the ether, but the blood-pressure maintained because of vaso-constriction and because the output of adrenaline and the vaso-constriction improve the coronary flow. During prolonged anæsthesia when the blood-pressure falls, the fall indicates that the limit of compensation for the cardiac weakness is reached. When a weakened heart is trying to maintain its output through maximally constricted vessels, there is likely to be a deficient peripheral circulation and resulting anoxæmia. It may be this which is the cause of the greatly increased sensitiveness to histamine demonstrated by Dale [1920] after ether or chloroform anæsthesia.

We think that the observations of Macdonald and Schlapp [1926] that doses of adrenaline which are pressor in the decerebrate cat become depressor when ether is administered, are explained by the action of ether in raising arterial tone; the depressor action of adrenaline will predominate when, on account of vaso-constriction, the pressor effect cannot develop. Macdonald and Schlapp found that urethane had the same effect as ether; we were not able to show that urethane caused immediate contraction of the spleen, but Elliott showed that it caused discharge of adrenaline from the suprarenal glands; hence urethane also may cause vaso-constriction. Chloroform certainly has the same qualitative action as ether.

In stating that the evidence indicates that the effect of ether on the vessels is to produce vaso-constriction by means of sympathetic impulses, we do not, of course, exclude the possibility of vaso-dilatation also produced by sympathetic impulses. One of us [Burn, 1932] has recently added to the evidence for a generalized system of sympathetic vasodilators in the dog, and it is probable that there is a similar system in the cat. If so, active sympathetic stimulation by ether may lead to dilatation in some parts of the vessels; this was in fact observed [Burn, 1925] when ether was administered to a cat under urethane. It may be that under urethane the vaso-constrictor fibres are already in full activity, so that the administration of ether leads to vaso-dilatation, just as under urethane doses of adrenaline, which are pressor in the decerebrate cat, become depressor. No confident explanation can yet be given of this dilator effect of ether, and the main effect of ether on the blood-pressure is that due to vaso-constriction.

SUMMARY.

1. Ether stimulates the sympathetic system as shown by the following observations on decerebrate or spinal cats from which the suprarenal glands have been removed; ether causes (a) contraction of the spleen; (b) immediate inhibition of the intestine; (c) inhibition of the uterus of the virgin cat; (d) rise in heart rate.

2. The stimulus is applied within the central nervous system; for the effects are not seen in the fully pithed animal, although a gradual intestinal paralysis may occur.

3. The effect on the spleen in the decerebrate animal is abolished by nicotine.

4. Ether usually has little effect on the blood-pressure of the decerebrate cat without suprarenals, though it often causes an initial rise; after nicotine it causes a steep fall.

5. When administered to the heart-lung preparation, ether greatly weakens the action of the heart; its effect is less than that of chloroform, but is much more than is commonly supposed. The weakening of the heart does not result in a fall in blood-pressure in the intact animal because of the rise in arterial tone and increased output of adrenaline. There is no evidence that ether depresses the vaso-motor system.

6. Ether has no effect on the vessels of a limb perfused with blood.

7. Chloroform and urethane have the same action as ether.

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RHYTHMIC ACTIVITY IN SKELETAL MUSCLE FIBRES.

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THESE observations deal with certain features of the rhythmic discharge which may be set up in striated muscle by non-rhythmic stimulation or treatment with various salt solutions. They were made in the hope of finding evidence which would apply to such discharges in general and particularly to those occurring in the central nervous system. Rhythmic activity in muscle has much the same character as that in sensory end-organs and in nerve cells, and muscle discharges have the advantage that they can be recorded at or near their point of origin. A disadvantage arises from the fact that in muscle there is a mechanical as well as an electrical response. This has introduced some uncertainty, but we have found several unexpected features in the muscular discharge and some indication of the conditions which produce spontaneous activity.

Since we have had to record slow as well as rapid changes, we have used a direct coupled amplifier leading to a Matthews's oscillograph. The arrangement is that described by Adrian [1931] with slight modifications.

No great sensitivity is needed to record action currents in single muscle fibres, and as a rule the amplifier has been arranged to give at maximum sensitivity a deflection of 10 mm. for 25 microvolts. A tapped volume control gives various fractions of this sensitivity, and it is usually set to give $1/4$ or $1/10$. With a metallic resistance in the input circuit there is no appreciable drift of the base line at maximum sensitivity. With the electrodes and preparation in circuit slow drifts appear from time to time, *e.g.* when the composition of the fluids is altered, but these are rarely troublesome. Glass U-tube electrodes were used, with silver, silver chloride leads. These must be shielded from light, as an unequal illumination produces a slow change of potential. A condenser coupling was used in some experiments in which it was necessary to eliminate slow changes. The usual rotating mirror and loud-speaker arrangements were used.

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Rhythmic discharges in NaCl solution. The twitching which occurs in a frog's sartorius bathed in 0.6 p.c. NaCl solution is accompanied by action currents of the familiar type, and as these are readily amplified it is much easier to follow the course of the activity by electrical than by mechanical recording. To secure continued action the muscle must be bathed in a large volume of the solution; if the volume is too small (as when the muscle is suspended in air on the electrodes) the activity soon ceases, but it is renewed by irrigation with fresh solution. The work of Dulière and Horton [1929] makes it likely that this is due to a diffusion of K from the muscle fibres: unless the excess is removed

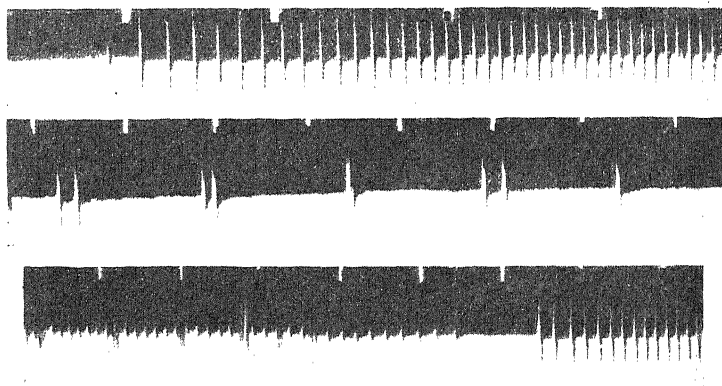


Fig. 1. Spontaneous discharges in frog's sartorius muscle after immersion in 0.6 p.c. NaCl solution. Records made with direct-coupled amplifier and Matthews's oscillograph. The action potentials are due to single muscle fibres. Time marker gives intervals of 0.25 sec. in this and all records.

the activity comes to an end. By making use of this effect it is easy to secure conditions in which there are only one or two series of impulses at a time, *i.e.* only one or two units in action. With frequent renewal of the solution spontaneous activity may continue for an hour or more, each renewal causing a fresh outburst which declines slowly.

Typical records of the discharges are given in Fig. 1. The muscles lay in a bath divided by a slot to form two electrodes, or were suspended in air on U-tube electrodes with cotton-wool plugs soaked in the solution. They were under enough tension to prevent visible movement but were not interfered with except during irrigation. The units responsible for each series of action potentials are, in most cases, the individual muscle fibres, for the discharges occur in curarized muscle, and in normal muscles

some of them are found to arise from the pelvic end beyond the region of nerve endings. The magnitude of the potential varies from one series to another, but the average size is of the order to be expected from a single muscle fibre.

The sartorius contains about 400 fibres and the whole muscle gives a potential (monophasic) of about 40 mv. Thus the average potential from one fibre should be 0.1 mv. [Watts, 1924]. The values recorded range from about 0.01 to 0.3 mv. The variation is probably due in part to the greater short circuiting of the fibres in the interior of the muscle.

The frequency of the impulses in a single series may range from about 1 to 80 a sec. (at 15° C.). The high-frequency discharges usually last for a much shorter time than the low-frequency, though the duration varies widely with the condition of the muscle in regard to irrigation. Discharges which attain a high frequency usually cease when the frequency has fallen to 10–20 a sec. Grouped discharges sometimes occur, each group made up of two or more impulses closely spaced. When several units are in action each preserves its own rhythm and there is no tendency to synchronization.

The behaviour of a muscle in NaCl solution is strongly reminiscent of that observed in the isolated nerve ganglia of insects [Adrian, 1930*b*, 1931]. These give spontaneous discharges with the same mixture of slow and rapid rhythms, which rise and decline in much the same way. The activity is often increased by irrigation with Ringer's fluid and it seems likely that the irrigation acts, as with muscle, by removing something which diffuses from the cells and tends to check their discharge.

The most significant evidence as to the cause of the activity in NaCl solution is that given by Mines [1908]. He found that a muscle in 0.6 p.c. NaCl was much more excitable to currents of long duration than one in Ringer's fluid, though to currents of short duration there was no difference. Thus in NaCl there is a much less rapid adaptation to an electric stimulus: as a result a weak constant current might produce a continued excitation and a repeated discharge of impulses. Potential gradients due to inequalities in the surface of the fibres might form the necessary stimulus. But eventually a stimulus of this kind might be unnecessary, for if there is normally an equilibrium between a reaction tending to produce the state of excitation and one tending to counteract it, a failure of the latter would be enough to start a discharge.

Mechanical stimulation. Stretch. When a fresh muscle has been for some minutes in NaCl solution a light touch on its surface or a slight

stretch will often produce considerable activity. When it has become sensitive to very slight mechanical stimulation of this kind there are usually a good many fibres discharging spontaneously; as these confuse the record we have usually worked with the muscle in a less excitable state. It is also necessary to use a condenser coupling in the amplifier to minimize potential changes of long duration.

The response to moderate stretch may then take several forms, illustrated in Fig. 2. Rapid discharges may start in one or more fibres

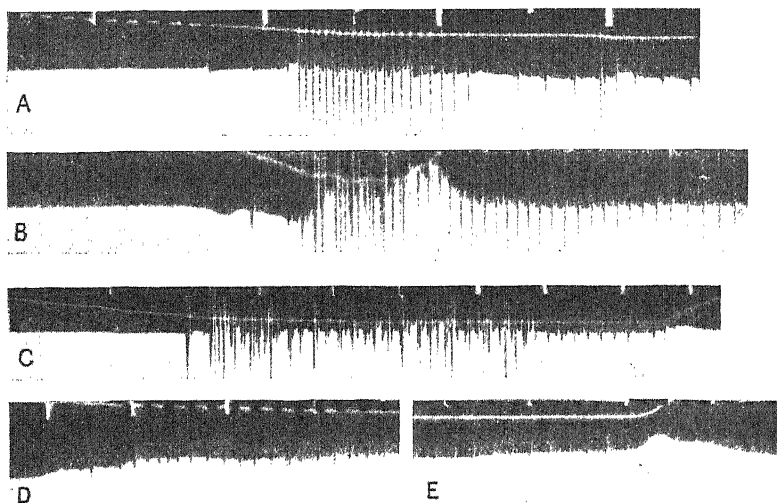


Fig. 2. Discharges produced by stretching muscles treated with NaCl solution. The stretch is shown by the white signal line. In A one fibre ceases to discharge before the stretch is relaxed; in B one begins to discharge at relaxation. In C, D and E, relaxation stops the discharge. D and E are from the same record with an interval of 2 sec. between them. Condenser coupling in the amplifier to eliminate steady potential changes.

and these may come to an end whilst the stretch is still in being (Fig. 2 A) or may persist for a variable time after it is over without any marked change of frequency at relaxation. The relaxation sometimes causes a fresh discharge (Fig. 2 B). But it is a common experience to find the discharge in some fibres persisting throughout the stretch and ceasing abruptly at relaxation (Fig. 2 C, D and E). The amount of extension needed to start the discharge varies with the state of the muscle, but that due to a weight of 1-2 g. is usually enough.

The result is chiefly remarkable in showing that a muscle fibre after treatment with NaCl solution behaves in much the same way as a sensory

nerve ending. A stretch receptor (*e.g.* a muscle spindle or a vagal ending in the lung) gives a rhythmic discharge on extension, and the chief difference is that with the sensory ending the frequency varies smoothly with the stretch, whereas the muscle fibre discharge shows much less flexibility. A spontaneous discharge of low frequency sometimes becomes more rapid when the muscle is stretched, but usually the frequency rises and declines abruptly and once the discharge has started an increase in the stretch has no further effect on it. This lack of grading is not surprising. The sole function of the sensory end-organ is to translate a stretch into an impulse message of graded frequency and its mechanism must work as flexibly as possible. The muscle fibre has no such function: if it is to be under complete control by the nervous system it must not react on its own account to a moderate stretch, and normally it does not. By treatment with NaCl it can be made to do so, but it is small wonder that the mechanism is jerky and inflexible.

Some idea of the mechanism may be gathered by considering the different kinds of discharge produced by stretch. The commonest type is the discharge which, once started, runs its course without further regard to the stimulus, ceasing before or after relaxation and showing no change of frequency when relaxation occurs. Here the exciting factor seems to be an injury which is perhaps healed by time but is not healed by relaxation of the stretch. An injury such as that caused by cutting or tearing the fibre gives a long discharge in NaCl, and the electric forces at the injured region would be an adequate stimulus. Clearly a transient injury would account in the same way for the discharges which cease at relaxation. A breakdown of the polarized surface might repair itself when the mechanical strain is removed, and if the breakdown involved complete depolarization the discharge would not vary in frequency with the stimulus. If the sensory ending has the same kind of mechanism we must imagine that its surface is much less rigidly constructed and so can be made to give a graded change of polarization according to the amount of mechanical strain. It may be noted here that the discharge of a stretch receptor agrees with that of a muscle fibre in regard to the action of salts, for immersion in NaCl prolongs the discharge and there is something which diffuses from the tissues and inhibits activity if the fluid is not changed from time to time [Matthews, 1931].

If it is true that mechanical stimulation acts by causing a local injury or depolarization of the fibre, we might expect to find evidence of steady potential changes due to the stretch. Unfortunately it is not possible to localize the effect of a stretch so that the discharge may be trusted

to start from the neighbourhood of one of the electrodes. There are considerable potential changes between the electrodes, sometimes coinciding with the extension and sometimes outlasting it, but so many factors might produce them that the observation has little value.

A possible method of testing the point seemed to arise from the fact that a discharge can be produced by localized pressure. Experiments with this form of stimulation are described below.

Stimulation by pressure. The sartorius was set up in a moist chamber with the tibial end in contact with the input electrode. The pelvic half of the muscle lay on a small glass plate with its upper surface in contact with the earthed electrode. This had a rigid end which could be pressed against the muscle to give the necessary stimulus.

It was made from a small block of vulcanite with a hole drilled vertically through it and plugged with a fragment of unglazed porcelain, sealed in with wax. The projecting end of the porcelain was ground flat so that it would lie evenly on the muscle. The hole was filled with NaCl solution and a spiral of silver wire coated with silver chloride was dipped into it from above. The electrode could be pressed on to the muscle by a weighed lever or by a positive screw movement.

A typical record, made with low amplification, is given in Fig. 3 A. The muscle had been bathed in NaCl, but was quiescent before stimulation. The movable electrode was held so that it made electrical contact with the muscle but exerted no pressure: it was then gradually pressed down by a fine screw adjustment. The result is a rapid fall of potential (indicated by the upward movement of the base line) and an outburst of impulses in many fibres. The pressure has produced an injury potential and the discharge is presumably set up by this.

With this form of stimulation we have not succeeded in recording a reversible excitation, a discharge which subsides as soon as the pressure is removed. There is often a slight reduction of the injury potential, but some permanent damage has always been produced, and so many fibres remain in action that it is difficult to be sure that any cease discharging when the pressure is relaxed. All that can be said is that in a muscle previously uninjured there is no sign of any production of impulses by pressure before the first appearance of the injury potential, no sign, that is, of a discharge which is not associated with a local depolarization of the fibre. The results are more erratic if the muscle has been damaged by previous stimulation and the fall of potential may then be masked by changes due to the movement of the region already injured.

But the records, although not as conclusive as we had hoped, have shown another feature of greater interest. This is illustrated in Fig. 3 B,

C and D. As the injury potential increases the discharge may suddenly change its character. The small, irregular fluctuations give place to large regular waves with a potential of several millivolts. Their size shows that they are due to a number of muscle fibres acting synchronously; the number cannot well be less than 10 and must often be 20 or more. The degree of synchronization varies, for the form of the waves may differ little from that of the action potential of a single fibre or it may

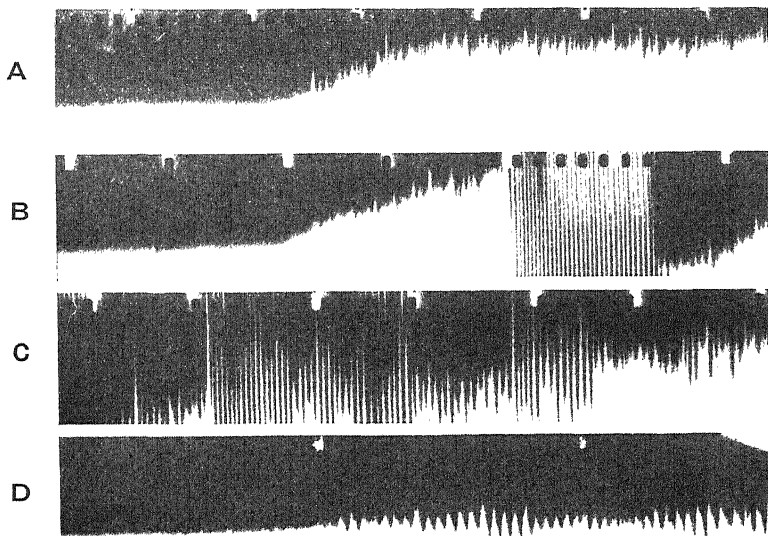


Fig. 3. Discharges due to pressure applied by one of the electrodes. Direct coupled amplifier. An upward movement of the base line denotes a fall of potential at the electrode which is pressed on the muscle. The discharge does not begin until some injury potential has developed. In B, C and D, large synchronous waves take the place of the smaller oscillations. In D the sensitivity is very much reduced, and the speed of the film increased to show the form of the waves.

be more like a smooth sine curve. The waves only occur at high frequencies: the maximum has varied from 90–120 a sec. and the minimum has never been less than 60. When the frequency has fallen to this value the discharge breaks up into the usual rapid, irregular type, though an occasional large wave may appear from time to time.

The synchronization of the muscle fibres is not due to the stimulus acting *via* the nerve, for it occurs after curarization and the pressure may be applied at the pelvic end beyond the region of nerve endings. Treatment with NaCl is not essential, for synchronous waves can be

produced in a muscle irrigated with Ringer's fluid, though the discharge lasts for a much shorter time. Evidently the phenomenon is characteristic of injured muscle and is closely related to the synchronization which occurs in injured nerve fibres [Adrian, 1930*a*]. In the latter the frequency of the waves is naturally higher (300–500 a sec. at 37° C.), and they may occur either at the moment of injury or some time after it, from slight changes of temperature, irrigation, etc. It has been argued that the synchronization in nerve is brought about by the electrical forces at and near the cut ends of the fibres and it is most likely that the waves in muscle have the same explanation. The essential conditions seem to be (a) an intense and fairly uniform activity in several fibres, and (b) the existence of damaged or permeable regions at neighbouring points in them. These would bring the interiors of the fibres into free electrical communication and give a chance to the group to behave as a single unit. An active region developing in one fibre close to the injury would tend to activate not only the neighbouring points in the same fibre but also those in the fibres next to it, and thus a rhythmic discharge in one fibre might come to dominate the rhythm of its neighbours. Erlanger and Blair have shown recently [1932] that this is more than a theoretical possibility, for they find that although in an uninjured fibre a wave of activity has no tendency to excite other fibres, there are distinct signs of interaction if the fibres are damaged.

The synchronization in these muscle and nerve discharges is of interest from the light it may throw on the synchronization which occurs in groups of nerve cells. Synchronous activity has been found in motor discharges, [Gasser, 1928; Adrian and Bronk, 1928], in the retina [Adrian and Matthews, 1928] and in the optic ganglion of insects [Adrian, 1932]. The usual conditions for its appearance are that the excitation should be uniform and of high intensity. In muscle and nerve we have to add the condition that there should be a steady depolarization at neighbouring points in the fibres; we may expect, therefore, that a similar depolarization occurs at some point in strongly excited dendrites or nerve cells.

Changes at the point of origin of the discharge. The activity caused by severe pressure is certainly associated with a persistent depolarization of the fibre at the stimulated point. That caused by stretch, and ceasing when the stretch is over, seems likely to be due to a reversible depolarization, but the evidence is not conclusive. The discharges which occur spontaneously are often indistinguishable from those due to mechanical stimulation and the high frequency discharges might well be due to a

sudden breakdown in some part of the fibre. But many of the spontaneous discharges have a very low frequency and it is unlikely that these can be explained in the same way unless there can be minor degrees of breakdown with a very slight stimulating effect.

The general state of polarization of the fibre surface (apart from localized breakdown) cannot be responsible for the tendency to activity, for some of the solutions which produce it increase, and some decrease the potential difference between an intact and an injured region. In NaCl, for instance, the injury potential is slightly greater than in Ringer's fluid, and if the fibre is uninjured a region bathed in NaCl is a few millivolts positive to a region bathed in Ringer. On the other hand solutions of 0.6 p.c. Na citrate, tartrate or oxalate cause great

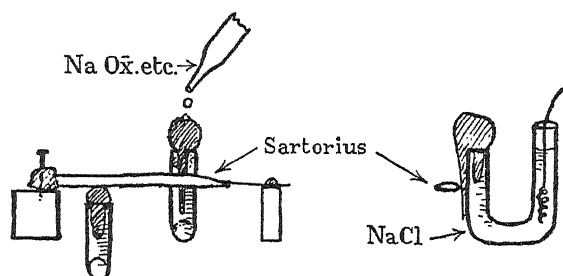


Fig. 4. Arrangement used for recording potentials developed at the point of origin of the discharge, showing method of applying salt solutions to the muscle by way of the electrode.

spontaneous activity, but a region bathed in them is negative to a region bathed in Ringer. It is probable, however, that there is the common factor of a slow rate of adaptation, for with all these solutions the concentration of calcium ions will be less than in Ringer's fluid. With all of them, therefore, the reaction which opposes the development of the excitatory state is likely to take place more slowly than in normal fibre, and in the end the excitatory state might develop from the mere absence of the opposing factor.

To collect more evidence as to this it seemed essential to record the electric changes occurring at the point of origin of the discharge. An application of NaCl solution limited to a few millimetres of the fibre cannot be relied on to cause spontaneous activity, but solutions of Na citrate, tartrate or oxalate will do so. After various trials we adopted the simple arrangement shown in Fig. 4. The sartorius is suspended horizontally and makes contact with two U-tube electrodes. These are

filled with 0.6 p.c. NaCl solution and plugged with cotton wool. The plug on the "active" electrode is pulled out into a tail which is turned down so that it lies against the side of the glass tube and touches the side of the muscle. Solutions dropped from a pipette on to the upper part of the plug flow down by way of the tail and drip off the bend in the U below; thus the fluid in contact with the muscle can be changed in composition with very little disturbance, and only a small length of the fibre is exposed to it. With this arrangement the addition of a few drops of 0.6 p.c. Na citrate, tartrate or oxalate at the top of the plug

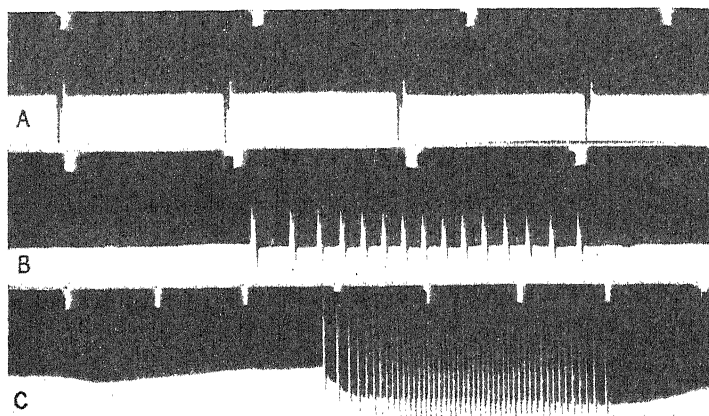


Fig. 5. Control records, made with direct-coupled amplifier, to show the form of the action potentials when the discharge starts from a point beyond or between the electrodes. The potential changes develop suddenly from a horizontal base line. In C there is a movement of the base line due, probably, to the contraction.

is usually enough to start a discharge of impulses in several fibres and it can be stopped again by washing with Ringer's fluid. Control experiments were made by applying the solutions at points beyond or between the electrodes, and the control records differ so markedly from those with the solution on the electrode that there is no doubt of the localization of the effect.

Typical controls are given in Fig. 5 A and B. They show the usual diphasic action potentials rising sharply from a horizontal base line. The action potentials in a series are all alike, and as a rule the movements of the contracting fibre have no effect on the record. Fig. 5 C is unusual in showing a displacement of the base line, but it appears as a smooth curve on which the action potentials are superimposed.

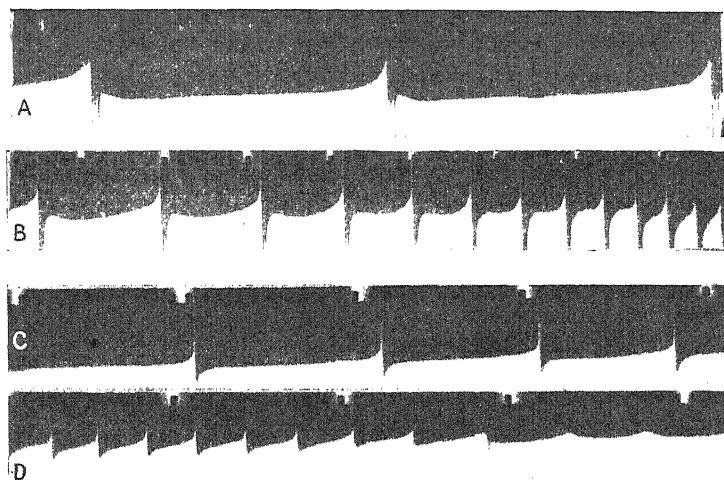


Fig. 6. Form of potential change when the discharge starts from one electrode. In A, C and D, 0.6 p.c. Na oxalate was applied to the electrode, in B, 0.6 p.c. Na citrate. An upward movement of the base line shows a fall of potential at the active electrode. There is a slowly developing fall of potential before each conducted wave and in D at the end of the discharge only the slow changes are left. C and D show the beginning and end of one discharge. Direct coupled amplifier.

Typical records made with the solution applied to the electrode are given in Figs. 6 and 7. The main difference from the controls is that there is now a series of slow potential changes, each leading up to one of the rapid diphasic waves. In Fig. 6 A the frequency is so low that the after effects of each wave are not likely to influence the form of the next, and each wave begins with a gradual fall of potential (shown by the rise of the base line) starting at least 0.1 sec. before the culmination of the wave. Fig. 6 C and D show the beginning and ending of a discharge; there is the same slow rise of the base line before each of the rapid deflections, and at the end the rapid deflections disappear leaving three or four slow waves which are evidently a continuation of those occurring throughout the discharge. The same result is shown in Fig. 7. The appearance of the slow waves alone at the end of a discharge is not a very common occurrence, but the slow change before each impulse is almost invariable. It occurs with Na citrate, tartrate or oxalate, and with 0.6 p.c. NaCl solution in muscles which will respond to a local application.

The records show other peculiarities, but the first point to decide is whether these slow changes of potential are due merely to movement of

the fibre or whether they indicate actual changes of potential at its surface. The fibre will certainly move when each impulse travels down it, but the potential begins to fall a long time before the conducted wave leaves the electrode. The fall of potential must therefore represent a slowly developing activity of some kind localized to the region of the active electrode. This may well involve both a contraction and a change of surface potential, but as the initial change is always in the same direction (a fall of potential at the electrode) we may reasonably conclude that it does imply a gradual depolarization of the muscle fibre.

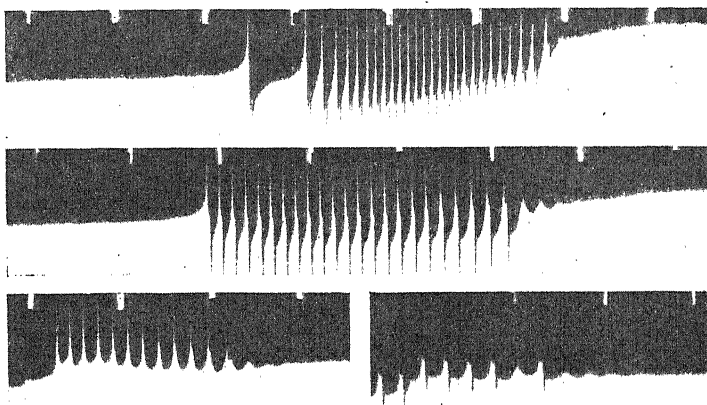


Fig. 7. High frequency discharges starting from one electrode (0.6 p.c. Na oxalate). The initial slow changes and the terminal oscillations are clearly visible. Direct coupled amplifier.

It is difficult to follow the course of the change at the active electrode after the impulse has passed down the fibre. Allowance must be made for the effects of movement, for the action potential at the distal electrode and for the electrical complications due to the presence of inactive tissue and fluid surrounding the active point. The latter probably explains, on the lines elaborated by Craib [1928], the appearance in some records of polyphasic excursions and the difficulty of obtaining pure monophasic potentials. To minimize the short circuiting effects of the inactive tissue we have made several experiments with thin strips cut from the side of the muscle and not much thicker than a frog's sciatic nerve. These are much less active than the whole muscle, owing, probably, to the substances which are constantly diffusing from the injured fibres. Records

from muscle strips and some from the whole muscle are given in Fig. 8 to show the variations in the form of the wave. In many preparations, after the initial slow rise of the base line has culminated in the spike of the action potential, there is a rapid decline to a lower level than before. In terms of potential this means that the active electrode becomes slowly and then rapidly negative to the distal electrode and

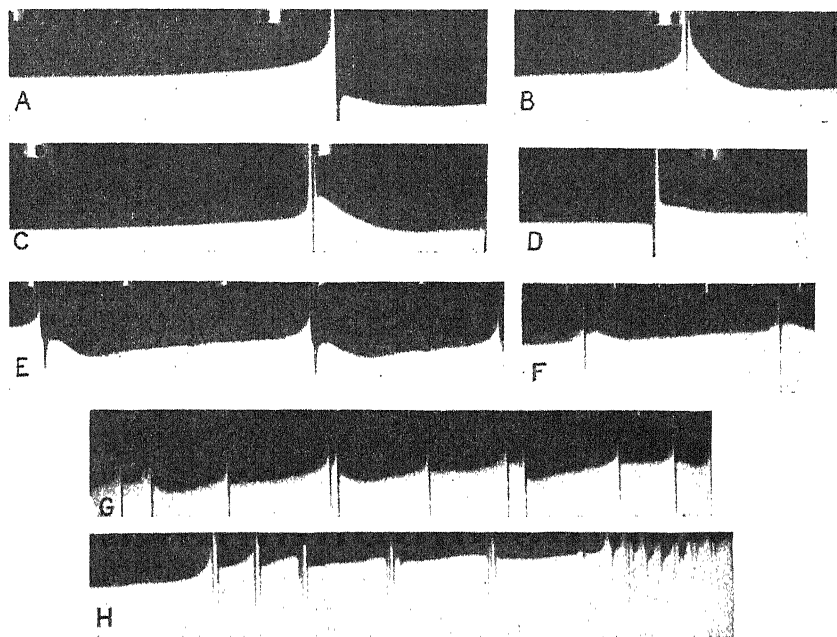


Fig. 8. Various forms of potential change when the discharge starts from the electrode. A, B and C are from a thin strip of the sartorius and are made at a higher speed than usual. D is from the same record, but the wave has started from the distal end of the strip and the gradual development is absent. E, F and G are from other preparations in which the form of the slow change is well marked. In H the waves are polyphasic. Direct coupled amplifier.

then more positive than it was at the start. It returns gradually to its previous value and the cycle is then repeated. In others there is no positive after effect and the negativity subsides slowly. The impulse may be discharged at the peak of the wave or near its beginning. Occasionally a single slow wave is associated with two or three impulses very closely spaced.

It is impossible to say how far the contraction and relaxation of the

fibre influence the exact shape of the wave. In normal muscle the rapid decline of the action potential is followed by a long tail, coinciding in time with the mechanical response [Bishop and Gilson, 1927], and the tail may be larger in the conditions of these experiments. At low frequencies the decline of each slow wave often looks suspiciously like an effect of mechanical relaxation, but at the end of a rapid discharge there is usually nothing to correspond to the relaxation after a tetanus. In Fig. 7 for instance there is no fall after the waves have ceased, though their frequency (15 a sec.) should have been high enough to give a summated contraction. The same uncertainty applies to another prominent feature of some records, a tendency for the rapid discharge of impulses to be accompanied by a fall of mean potential. With this there is a progressive decline in the size of the upward spike, though the downward spike (*i.e.* the potential change at the distal electrode) remains unaltered and disappears suddenly. A progressive depolarization of the fibre under the active electrode would account for these changes, but the fall of potential does not always take place. In the control record, Fig. 5 C, there is a fall of potential at the distal electrode which, from its contour, is clearly a mechanical effect, and the same kind of effect may account for some of the changes mentioned above.

It is clear that a tissue which responds mechanically is not the most suitable object for electrical analysis. But even if we leave out all but the initial changes of potential the results are suggestive enough. They imply that at the point of origin of the rhythmic discharge the activity develops much more gradually than in the normal part of the fibre. A general slowing of the whole mechanism can scarcely account for this, for the impulses in a spontaneous discharge may have frequencies as high as 80 a sec. The maximum frequency at which a normal fibre of the frog's gastrocnemius can respond to indirect excitation is about 150 a sec. (at 15° C.) and that for direct excitation cannot be far different. But a twofold increase in time relations would not be nearly enough to explain the slow development. The most likely explanation is that it is a consequence of the slow rate of adaptation. In the absence of Ca^{++} the reaction which opposes excitation is slowed down and a gradual building up of the active state becomes possible. The activity which is expressed by the fall of potential is probably the culminating event in a series of reactions, for at very low frequencies the potential may remain steady for the greater part of the interval between successive waves. This agrees with the finding that in a fragment of frog's auricle the potential does not rise or decline appreciably between the beats [Adrian, 1931].

Something which leads to excitation must be taking place in these intervals, but it is only towards the end that changes of potential become evident. For this reason we can scarcely equate the development of the potential change with the building up of the "excitatory state" in the sense used by Eccles and Sherrington for the rhythmic discharge of nerve cells, though no doubt the two are closely related.

It is interesting to find that at the point of origin of the muscle discharge the potential changes develop much less explosively than in the normal fibre, for slow potential changes, rhythmically oscillating or steady, are met with in the central nervous system, and the muscle records can often be paralleled very closely by records of the activity in groups of nerve cells (cf. Adrian, 1932). But this may be no more

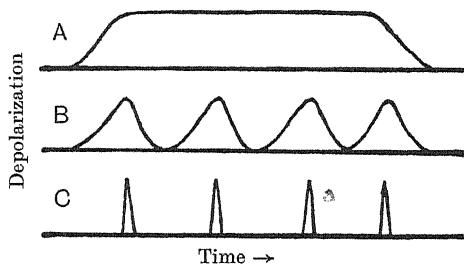


Fig. 9. Three possible types of electrical activity. C is characteristic of normal muscle and nerve fibre. It is suggested that both A and B may occur at the point of origin of rhythmic discharges.

than a casual likeness, and it would be a waste of time to discuss it until the same result has been found with a preparation which does not respond mechanically.

The conclusion must be tentative and it is summarized briefly in Fig. 9. It is suggested that the electrical activity involved in a rhythmic discharge may be of three types, (1) a steady depolarization like that due to permanent or transient injury, (2) an oscillation between the polarized and depolarized condition, and (3) an abrupt depolarization with an almost immediate recovery. In the normal muscle or nerve fibre only the third kind of activity is possible (apart from injury). A rhythmic discharge may be evoked by injury, but the adaptation to the stimulus makes it rapidly ineffective. But with a slower rate of adaptation the active state may rise less abruptly, it may need a smaller stimulus to evoke it, or even no stimulus at all, and a steady stimulus

(*e.g.* a steady depolarization at some point) will cause a persistent oscillation between rest and activity. By local treatment of a muscle with solutions which remove calcium we can produce a slowly adapting region of this kind, and it seems quite probable that similar regions may exist in nerve cells, dendrites and sensory endings.

SUMMARY.

1. Records have been made of the electrical activity of the frog's sartorius muscle treated with NaCl and other salt solutions which lead to spontaneous activity. The action potentials in the individual muscle fibres appear in rhythmic discharges like those from sensory nerve endings and from nerve cells.

2. Stretching the muscle (in NaCl solution) may excite some of the fibres, but in comparison with a sensory nerve ending the muscle fibre is a much less flexible receptor.

3. Stimulation by pressure gives a local depolarization and a discharge of impulses. At the height of the discharge the different muscle fibres may begin to respond synchronously. The synchronous discharge is compared with that from injured nerve fibres and from groups of nerve cells.

4. Spontaneous discharges may be produced by local application of Na citrate, tartrate or oxalate. When solutions of these are placed on one of the electrodes the potential changes can be studied at or near the point of origin of the discharge. They differ considerably from the potential changes in other parts of the fibre, beginning always with a very slow fall of potential which leads up to the rapid conducted wave. At the end of a discharge the conducted waves may fail, leaving only the slow, local oscillations.

It is concluded that a gradual development of the active state is made possible by a slowing of the rate of adaptation in the muscle fibre. The decline of the active state cannot be followed with certainty owing to the movement of the fibres.

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GLYCOGEN SYNTHESIS IN THE SMALL INTESTINE.

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It has been claimed that the glycogen content of the portal blood increases during absorption of carbohydrates (see Verzar for references, 1932); but Charit [1926] was unable to obtain confirmatory evidence in angiotomized dogs. Lang [1928] criticized Charit's methods, and published findings which seemed to show that the glycogen in portal blood is increased to a variable extent in anæsthetized dogs during absorption of glucose and fructose. With glucose he found increases of 0.9–24 mg./100 c.c. of blood, with an average of 9.5 mg. in six experiments. He, therefore, concluded that the intestinal wall builds up glycogen and passes it into the portal blood during absorption of carbohydrates.

Verzar [1932] has made use of these results to support his argument that the phenomena of absorption can be interpreted in physico-chemical terms. He maintains that the fundamental basis of the selective absorption of glucose, as compared with foreign sugars such as xylose, is an unspecified transformation produced in the glucose as soon as it enters the epithelium. The glucose-diffusion gradient from intestinal lumen into cell is thereby kept at a high level, so that diffusion can proceed until no more sugar is left in the lumen. Verzar, assuming that Lang's conception is correct, makes no attempt to explain in terms of his physico-chemical theory of absorption why the mucosa either transforms only part of the absorbed glucose into glycogen; or, if it transforms all of it, why part of this newly-formed glycogen is again hydrolysed to sugar while the other part makes its way into the blood as glycogen. It appeared to us a more logical hypothesis that the absorbed glucose is entirely synthesized into a temporary labile form of glycogen and that it is rehydrolysed into sugar in order to pass through the attached border of the epithelium and the walls of the capillaries. This hypothesis is analogous with Loevenhart's hydrolysis-resynthesis theory of fat transport.

The object of the present research was to test this conception of carbohydrate absorption. It is clear, however, that Lang's results are incompatible with such a view, and a repetition of his experiments was obviously necessary at the outset.

Blood glycogen during glucose absorption. In dogs under urethane or ether the splenic artery was tied, blood massaged out of the spleen, the vein ligatured and a cannula fixed in the proximal end. One or two samples of blood (30 c.c.), depending on the size of dog, were then run into tared flasks containing 60 p.c. KOH and the weight of blood determined. Forty c.c. or more of 0.75 *M* glucose were then injected into the duodenum and as many blood samples (30 c.c.) as possible taken after 15 min. or more. Glycogen was estimated by Pflüger's method. Great difficulty was experienced in getting rid of the pigments, repeated precipitations and filtrations being necessary. In a preliminary test of the method, 3.63 mg./100 g. were found in the jugular blood of a dog, assuming that all the reducing substance found after hydrolysis of the alcohol precipitate was glucose. The assumption could not be confirmed experimentally because of the very small amount of reduction. It is noteworthy that the above value falls within the range found for blood (1.5–6.1 mg./100 c.c.) by Huppert [1894] and by Schöndorff [1903].

The glycogen content in the portal blood of four dogs was determined before and after injection of glucose into the duodenum. In two, no glycogen was found in any of the samples, and in one, none in the first sample and traces only 15 min. after giving the glucose. In the fourth dog the following data were obtained: before giving glucose, 3.55 mg.; 30 min. after, 2.67 mg.; 60 min. after, 2.33 mg./100 g. We formed the opinion from these results that glycogen is present in blood in such minute amounts that estimation of it with any degree of accuracy is wellnigh impossible. For this reason we think that very little significance can be attached to the small increases observed by Lang. Furthermore, it is very improbable that a colloid like glycogen could diffuse through cell membranes into the blood. As further research along the above lines appeared pointless, experiments were carried out to determine whether the glycogen content of the intestinal wall is increased as a result of the passage through it of glucose, either by diffusion in the case of surviving intestine, or by absorption in intact animals. In the earlier experiments the glycogen in the entire intestinal wall was estimated; but, later, the technique was modified so that estimations on the dry mucosa alone were possible.

Glycogen content of intestinal wall during glucose absorption; (1) *surviving intestine*. Intestinal loops were taken from rabbits fasted 15 hours, suspended in oxygenated Tyrode at 40° and 4–6 c.c. 0.75 *M* glucose or 0.9 p.c. NaCl put in adjacent segments. The order of the loops in which the solutions were inserted was varied, so as to allow for possible physiological variations in different levels of the intestine. At intervals after putting in the solutions adjacent loops were removed from the bath, slit open, dried between filter papers and transferred to warm KOH as rapidly as possible for glycogen estimation. The amounts of glycogen, expressed in g./100 g. of fresh tissue, were compared with those obtained for control segments which were digested in KOH immediately after excision.

As can be seen from the examples of results given below, the surviving intestine showed no indication of ability to build up, and temporarily store, glycogen. In the intestines of different animals large variations were found which were probably due, to some extent, to variations in the moisture content of the intestinal wall. In those segments with high initial values fairly rapid glycogenolysis was usual as Exp. II shows. To avoid this complication the remaining experiments were performed on living animals.

Glycogen in rabbit's surviving intestine.

	Solution in loop	Diffusion time (min.)	Glycogen mg./100 g.
Exp. I	Nil (control)	0	8.1
		0	8.4
	0.75 <i>M</i> glucose	30	6.8
	0.9 p.c. NaCl	30	5.9
	0.75 <i>M</i> glucose	60	2.4
	0.9 p.c. NaCl	60	1.1
Exp. II	Nil (control)	0	25.6
		0	30.0
	0.75 <i>M</i> glucose	15	17.0
	0.9 p.c. NaCl	15	13.3
	0.75 <i>M</i> glucose	45	13.5
	0.9 p.c. NaCl	45	15.0

(2) *Anæsthetized animals*. In rabbits under urethane the small intestine was divided into two portions of approximately equal length, by means of ligatures. Into one portion 20 c.c. of 0.75 *M* glucose were injected, and into the other 20 c.c. of iso-osmotic NaCl solution (2.66 p.c.). The abdomen was sewn up and, an hour later, the segments were taken out and the glycogen in the entire wall determined. Again, no sign of an increase in glycogen in the glucose-absorbing portion was found. In virtue of these results, the estimations in succeeding experiments were done on the mucosa alone. The procedure was to excise the loops at the

end of the absorption period, and then rapidly fill them with absolute alcohol. This precipitated the glycogen and fixed the mucosa, so that it could be readily removed by scraping with a blunt scalpel. The mucosa was dried thoroughly by pressing between filter papers, or by heat at 100°, and the glycogen estimated in 0.5 g. samples by Osterberg's [1929] modification of Pflüger's method. The results were expressed as p.c. of dried mucosa.

From the typical results shown below it can be seen that the evidence as to glycogen synthesis was contradictory. They suggested, however, that the mucosa of the lower half was initially richer in glycogen than that of the upper half.

Glycogen in dried mucosa after absorption for 1 hour.

		Glycogen mg./100 g.	Difference
Av. of 3 exps.	Upper half containing glucose	110	30
	Lower half containing saline	140	
Av. of 2 exps.	Upper half containing saline	115	38
	Lower half containing glucose	153	

It thus appeared possible that an increase in glycogen in the duodenal half might be completely masked by comparison with the ileal half, when this was used as a control.

This opinion proved to be correct; for, when the glycogen in the mucosa from different levels in the fasted rabbits was determined, it was found to be significantly lower in the duodenum than at other levels. The average values in four animals were:

	Glycogen mg./100 g.	Average
Duodenum	354	404
Upper jejunum	454	
Jejuno-ileum	505	468
Ileum	432	

The glycogen in the lower half was 64 mg. higher than in the upper half. In the foregoing data the same relationship existed; but the differences were similar, 30 and 38 mg., in spite of the order being reversed in the respective experiments. It is clear therefore that these data do not provide any evidence of glycogen formation during glucose absorption. It was thought that the failure to obtain such evidence might be due to rapid breakdown in the (hypothetical) glycogen. In the succeeding experiments, therefore, the mucosa was fixed *in situ* in the animal, while the circulation was still proceeding.

The next series of experiments was done on cats under urethane, because the relatively short small intestine of these animals is believed to possess correspondingly powerful absorbing capabilities. An hour after injecting glucose into the duodenum, absolute alcohol was forced through the intestine, and then retained in it under pressure for several minutes. The intestine became gradually paler, and circulation through it stopped. It was then excised and the mucosa was scraped off. The mean glycogen values for the mucosa of two (control) cats, which received no intestinal injection, and for two cats, which received glucose in the upper half and nothing in the lower half, are subscribed:

	Controls	Glucose injected into upper half	
Upper half	735	718	Glycogen mg./100 g. mucosa
Lower half	832	824	

As these findings threw no additional light on the problem, the next series of experiments was carried out on unanæsthetized animals.

(3) *Unanæsthetized animals.* Rats which had fasted 24 hours were given by stomach tube 5 c.c. 0.75 *M* or 3 c.c. 3 *M* glucose, and stunned after an hour. The abdomen was rapidly opened, and the mucosa of the whole small intestine devitalized with alcohol, as described above. The blood was always circulating when the intestine was excised immediately afterwards. The glycogen values obtained for the mucosa of animals given 5 c.c. 0.9 p.c. NaCl instead of glucose were used as standards for comparison.

Glycogen in mucosa of rats.

No. of animals	Solution fed	Glycogen mg./100 g.	Difference
10	Glucose	163	23
10	Saline	140	

The average results show that the glycogen content of the mucosa of the rats given glucose was higher than that of those given saline by 23 mg. This difference appeared too small to be significant, and, to settle the point, the experimental error in the glycogen estimation was determined by analysing mixed samples of dried mucosa. The mean of six samples was 580 mg., the maximum 593 mg., and the minimum 565 mg. The experimental error was therefore ± 14 mg. As the above difference falls within this range it must be regarded as insignificant.

Microscopical examinations were also made of sections of intestine fixed during the absorption of either saline or glucose and stained by iodine. No evidence of glycogen accumulations could be found in the epithelium of the glucose-absorbing intestine.

We do not consider the results of this study as being a complete answer to the question we set out to answer, for two reasons. In the first place our knowledge of the chemistry of glycogen is far from satisfactory, and it is very probable that present-day methods of estimation are too inaccurate for studies of this nature. Or again, a polysaccharide of smaller molecular weight and more soluble than the compound precipitated by 60–70 p.c. alcohol, may be formed in the epithelium so that it would escape detection altogether. In the second place, it is conceivable that glycogen is built up and broken down again with such rapidity inside the epithelium that the normal content is not exceeded. Obviously, the methods we have employed could not provide an answer to this question. Indeed, problems of this type rarely yield to direct attack, but rather to the accumulation of circumstantial evidence. It is not, however, clear how even evidence of this kind can be brought to bear on this particular problem. The prospect is, however, more encouraging in regard to a similar hypothesis on which experiments are now in progress in this laboratory. This is based on experiments which showed that absorption of glucose was increased by the presence of phosphate at pH 7 [Magee and Reid, 1931]. The theory is that glucose combines with phosphate inside the epithelium to form hexose phosphate, instead of glycogen as in the former hypothesis. This (hexose-phosphate) theory is rendered all the more probable by recent work from Verzar's laboratory [Wilbrandt and Laszt, 1932]. These workers found that injections of iodoacetic acid (which inhibits formation of hexose-phosphate) apparently stopped selective absorption of glucose in anæsthetized animals.

The only conclusion which appears justifiable from this study is that, as judged by present-day analytical methods, a building-up of glycogen in the intestinal mucosa during glucose absorption is extremely improbable.

SUMMARY.

No evidence was obtained of an increase in the glycogen content of the portal blood of anæsthetized dogs during glucose absorption.

Glycogen determinations on the entire wall and the mucosa of the small intestine, either surviving or *in situ*, in anæsthetized rabbits and cats and in unanæsthetized rats, gave no evidence of formation of glycogen during absorption of glucose.

The glycogen content of the mucosa is lower in the duodenum than in other levels of the small intestine.

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THE RE-SYNTHESIS OF CREATINEPHOSPHORIC ACID
IN FROG'S MUSCLE POISONED WITH
IDOACETIC ACID.

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THE importance of creatinephosphoric acid in the cycle of chemical changes which provide the energy necessary for the development of muscular tension has become increasingly recognized since the fundamental work of Lundsgaard [1930*a*] showed that muscle poisoned with iodoacetic acid could perform a considerable amount of work without the production of lactic acid. Lundsgaard was able to show that such muscle depends upon the breakdown of creatinephosphoric acid as a source of energy, and subsequent work has made it clear that the breakdown of glycogen to lactic acid in unpoisoned muscle serves as an anaerobic source of energy for the re-synthesis of creatinephosphoric acid. If for any reason the production of lactic acid does not take place, there is no available anaerobic source of energy for the re-synthesis of creatinephosphoric acid except the small amount of heat produced by the breakdown of adenylypyrophosphate. This state of affairs exists in muscle poisoned with iodoacetate or fluoride [Lipmann, 1930] and to some extent if the carbohydrate store is diminished by starvation [Claude Bernard, 1877] or insulin [Ochoa, 1930].

In the case of aerobic muscle it was shown by Meyerhof and Boyland [1931] that muscle poisoned with iodoacetate had a respiratory quotient of 0.7, which was restored to a normal value of 0.95 by addition to the system of sodium *D*-lactate. This discovery led to the observation by Mawson [1932] that if sodium *D*-lactate was supplied to a muscle which was subsequently poisoned with iodoacetate the muscle would, in the presence of the lactate and oxygen, give a much longer series of twitches than would a poisoned muscle in presence of oxygen alone. It was shown that this prevention of the development of the symptoms of iodoacetate poisoning depended upon the presence of oxygen, and that

during the activity of the muscle lactate was removed from the surrounding medium.

It was suggested that the muscle was able to utilize the energy of combustion of added lactate for the re-synthesis of creatinephosphoric acid, and the conclusion was drawn that the non-appearance of lactic acid in poisoned muscle is the cause, rather than a symptom, of the loss of function which commences after 100-150 twitches. For the purposes of this argument it was assumed that the prolongation of irritability implied re-synthesis of creatinephosphoric acid, but no analytical evidence of such re-synthesis was brought forward. It has now become possible to produce evidence of this nature.

In the first experiments along these lines homologous gastrocnemius muscles of *Rana esculenta* were used. One muscle was soaked for 30 min. in oxygenated phosphate-Ringer solution and the other in a similar solution containing 0.05 p.c. sodium *d*-lactate. Iodoacetate was then added to both to a concentration of 1/20,000 and left for 30 min. The muscles were then simultaneously stimulated until the former became non-irritable and creatinephosphoric acid determined in both muscles. The results were inconclusive, probably because of the thickness of the muscles used and the consequent difficulty of diffusion of lactate and oxygen.

Similar experiments were then carried out using single pairs of sartorii of *R. esc.* and *R. temp.* set up as described by Mawson and Ritchie [1932]. In each experiment one pair of sartorii was stimulated to non-irritability in oxygenated phosphate-Ringer solution containing iodoacetate (1/17,500 to 1/25,000) and the other pair made to give the same number of twitches in a similar solution containing 0.05-0.10 p.c. sodium *d*-lactate. Creatinephosphoric acid was estimated by three different methods and in all cases it was found that the muscle which had received lactate, and was still irritable when analysed, contained considerably more creatinephosphoric acid than the non-irritable muscle which had received no lactate.

It was, however, surprising to find that the non-irritable muscles which had gone into rigor in absence of lactate contained (mean of twelve results) as much as 26.9 mg./100 g. of phosphorus as creatinephosphoric acid. Lundsgaard [1930*b*] observed that re-synthesis of creatinephosphoric acid takes place to some extent in poisoned muscles in presence of oxygen, for he found that whereas an anaerobic muscle which had been stimulated until it was non-irritable contained about 7 mg. of phosphorus in this form per 100 g., an oxygenated muscle which

had undergone similar stimulation was still contractile and contained about 46 mg./100 g. In the cases quoted in the present paper, however, the *oxygenated* muscle has been stimulated to non-irritability and the author is unaware of any previous observations on this point. Lundsgaard suggests that the "residual" creatinephosphoric acid varies with the time of year, so it may be useful to mention that the results recorded in this paper were obtained between November 7th, 1932, and January 23rd, 1933.

It was suspected that at least some part of the "residual creatinephosphoric acid" might be some other labile "soluble ester," so, on the suggestion of Prof. A. V. Hill, four experiments were done in which the Eggleton extrapolation technique was used [Eggleton and Eggleton, 1927]. Two experiments were done with added lactate and two without, but perfectly smooth curves were obtained which gave results similar to those obtained by the separation methods previously employed, so it seems likely that the residual substance was actually creatinephosphoric acid.

A few determinations of hexosemonophosphate were carried out, and it appears likely that the "non-lactate" muscles contained considerably more of this compound than the "lactate" muscles. In other experiments in which total phosphate was estimated the results were unusually high, perhaps owing to the use of phosphate-Ringer, but it seems that no significant difference exists between the two types of muscle in the total phosphorus of that fraction of compounds which is neither creatinephosphoric acid nor inorganic phosphate. This includes hexosemono- and -diphosphate together with adenylyl-pyrophosphoric acid, and will henceforth be referred to as "total esters."

EXPERIMENTAL.

In nearly all cases male *R. temp.* were used, as these proved to be the most suitable animals for this work. It was found convenient to use concentrations of lactic acid between 0.05 p.c. and 0.10 p.c., as this appeared to be the lowest order of concentration of lactate with which one could be sure of obtaining inhibition of the poisoning action of iodoacetate of a concentration as high as 1/20,000.

Experiments were carried out as described by Mawson [1932]. In most cases "lactate" and "non-lactate" muscles were used alternately under similar conditions in order to obtain as rigorous a control as can be achieved with iodoacetate poisoning. Lundsgaard [1930b] has pointed out the impossibility of obtaining two muscles poisoned in a

strictly similar manner, but the controls were adequate for the purposes of this work.

In the first series of experiments the separation method of Fiske and Subbarow [1929] was used for the estimation of creatinephosphoric acid, and throughout these experiments their method of phosphorus determination has been employed [Fiske and Subbarow, 1925]. It was found, however, that the crystallization of calcium sulphate in the final blue solution led to some inconvenience, and in the second series a modification of the Eggletons' method was employed [Eggleton and Eggleton, 1929-30]. This method depends upon the different solubilities of the barium salts of phosphoric acid, creatinephosphoric acid and the hexosephosphoric acids, and was found very convenient in operation. Instead, however, of neutralizing the deproteinized muscle filtrate with powdered baryta it was found to be more convenient, and equally effective, to neutralize with saturated caustic soda solution to a phenolphthalein end point [Fiske and Subbarow, 1929] and then to add one-quarter of the volume of 10 p.c. BaCl_2 solution containing 0.2 p.c. $\text{Ba}(\text{OH})_2$ and a trace of BaCO_3 .

Finally, four experiments were done in which the Eggleton extrapolation technique was used. As it is desirable here that colour development in standard and unknown solutions should commence at the same time, it is useful to place the sample and the standard phosphate in the bottom of standardized cylinders, carefully run in distilled water to as great a volume as circumstances permit, add the Fiske-Subbarow reagent and then the acid molybdate, and finally make up the last fraction of a c.c. to the mark with distilled water. If this is carefully done little acid will reach the creatinephosphoric acid solution and the two cylinders can be shaken up simultaneously. By the use of amino-naphtholsulphonic acid instead of hydroquinone these precautions are not so necessary, as the full colour is developed within five minutes, but they were taken in all cases.

Table I gives the results of several experiments using all three methods of estimation, and the mean of the results gives the creatinephosphoric acid content of the "non-lactate" muscle as 26.9 mg. P/100 g. of muscle and that of the "lactate" muscle as 46.4 mg. P/100 g. The inorganic phosphorus is the same in both cases and the phosphagen ratios are respectively 0.96 and 1.61. Addition of lactate, then, to the oxygenated poisoned muscle has given rise to a re-synthesis of creatinephosphoric acid containing 19.5 mg. phosphorus per 100 g. of muscle during a number of twitches which, without lactate, would have given rise to non-irritability and rigor.

TABLE I.

a. Without added lactate.

Exp. No.	Iodoacetate concentration	Phosphagen P mg./100 g.	Inorganic P mg./100 g.	Phosphagen ratio
206	1/20,000	25.6	28.6	0.90
216	1/20,000	24.9	34.9	0.71
220	1/20,000	29.3	27.0	1.09
221	1/25,000	31.1	26.2	1.19
224	1/25,000	28.2	24.3	1.12
Mean of 12 results (all methods)		26.9	28.1	0.96

b. With added lactate.

Exp. No.	Iodoacetate concentration	Phosphagen P mg./100 g.	Inorganic P mg./100 g.	Phosphagen ratio
207	1/20,000	55.2	43.4	1.27
211	1/17,500	47.0	28.9	1.63
219	1/20,000	47.7	23.8	2.00
222	1/25,000	44.2	15.8	2.80
223	1/25,000	59.6	30.3	1.97
Mean of 11 results (all methods)		46.4	28.9	1.61

Experiments 201-216 were done by the Fiske-Subbarow separation method, 217-220 by the modified Eggleton separation method, and 221-224 by the Eggleton extrapolation method.

Table II gives the results of experiments in which hexosemonophosphate was determined. After removal of inorganic phosphate and "insoluble esters" by the barium treatment the solution, containing hexose monophosphate and creatinephosphoric acid, was treated with

TABLE II.

Without added lactate		With added lactate	
Exp. No.	Monophosphate P mg./100 g.	Exp. No.	Monophosphate P mg./100 g.
216	58.9	215	49.4
218	60.8	217	28.4
220	65.3	219	31.8
Mean 61.7		Mean 36.5	

sulphuric acid, boiled down and phosphorus estimated according to the method of Eggleton and Eggleton [1929-30]. The hexosemonophosphate phosphorus was then calculated by subtraction of the value obtained for creatinephosphoric acid from another sample of the solution. The results show that the muscle which went into rigor without lactate contained 61.7 mg. P/100 g. of muscle in form of hexosemonophosphate while the "lactate" muscle contained only 36.5 mg./100 g.

When the experiments by the extrapolation method were being done the total phosphorus was determined in one portion of the deproteinized filtrate. By subtraction from this figure of the sum of the inorganic and the creatinephosphoric acid phosphorus a figure was obtained for what has been called "total esters." Table III shows that this sum is about the same in both "lactate" and "non-lactate" muscle.

TABLE III.

Without added lactate			With added lactate		
Exp. No.	Total esters mg. P/100 g.	Total P mg./100 g.	Exp. No.	Total esters mg. P/100 g.	Total P mg./100 g.
221	121.2	178.5	222	100.5	160.5
224	82.6	135.1	223	91.2	181.1
Mean	101.9	158.6	Mean	95.9	170.8

DISCUSSION.

The experimental results reported in this paper lend strong support to the suggestion that a muscle poisoned with iodoacetate can utilize the energy of combustion of added lactate for the purpose of re-synthesis of creatinephosphoric acid. The high values obtained, however, for creatinephosphoric acid in muscles which, in presence of oxygen but without lactate, had been stimulated until they were in rigor and would not respond to a continuation of the previous stimulation calls for some explanation. It would seem to indicate that the presence of creatinephosphoric acid is not in itself an indication of irritability, as indeed was shown by Dulière and Horton [1929]. In their case, however, the muscles were flaccid and would recover their irritability on soaking in Ringer solution, which is not the case with muscles poisoned with iodoacetate.

The observation that more hexosemonophosphate occurs in the "non-lactate" than in the "lactate" muscle tends to confirm the view that this compound is an "abnormal" stabilization product of Meyerhof's labile hexosemonophosphate [Meyerhof, 1926]. The muscle is in neither case producing lactic acid so that no more accumulation of "lactacidogen" would be expected for this reason in one muscle than in the other. The similarity of the quantities observed for "total esters" indicates that only the balance of proportionality between these compounds is altered by addition of lactate, and not their total amount. These proportions, however, may be of interest in view of the importance ascribed by Baldwin [1933] to adenylyl-pyrophosphate in the creatinephosphoric acid and lactic acid cycles. He regards adenylyl-pyrophosphoric acid as the

immediate source of energy for the re-synthesis of creatinephosphoric acid, and the figures given above suggest that there may be less adenylypyrophosphate in the "non-lactate" muscle than in the "lactate" muscle.

SUMMARY.

1. A frog's sartorius muscle, poisoned with iodoacetic acid, which has been stimulated until non-irritable in presence of oxygenated phosphate-Ringer solution, contains only about 58 p.c. of the creatinephosphoric acid present in a similar muscle which has received the same number of stimuli in a similar Ringer solution to which 0.05-0.10 p.c. sodium lactate has been added.

2. Results are reported which suggest that the hexosemonophosphate content of "non-lactate" muscle is greater than that of "lactate" muscle, while the reverse may be the case with the adenylypyrophosphate content.

The author wishes to express his gratitude to Mr A. D. Ritchie, under whose direction this work was carried out, and to Prof. H. S. Raper for their interest and helpful advice. He also acknowledges a grant from the Medical Research Council.

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ON THE SUPPOSED INHIBITORY ACTION OF THE
AURICLES ON THE AMPLITUDE OF THE
VENTRICULAR CONTRACTIONS IN
THE HEART OF THE FROG.

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VARIOUS workers have claimed that the auricles of the frog's heart exert an inhibitory action on the contractions of the ventricle. The observation which has led to this conclusion is, that removal of the auricles at the A.-v. groove gives rise to an increase in the amplitude of the ventricular contractions. There is, however, another change which always occurs when the auricles are removed, namely one of rate. The idio-ventricular rhythm which then supervenes is very much slower than the auricular rhythm, and it seems possible that this slowing of the rhythm may account for the increase in the amplitude of contraction. It has been shown by Hofmann [1901] that the ventricle of the frog gives maximal contractions at a rate of 12-15 beats per minute, the amplitude of the contractions being less at higher and lower rates. Now the natural rhythm of the frog's auricle is usually 25 beats per minute or more, and it is to be expected that a slowing of this rhythm, however it is produced, will cause an increase in the amplitude of the ventricular contractions. If the inhibitory action of the auricle really exists, the increase in amplitude of contraction produced by cutting off the auricle should still occur when a change in rate is excluded by artificial stimulation. If, on the other hand, the increase in amplitude is caused by the change in rate, it should be possible to produce the same effect in an isolated strip of ventricle artificially stimulated. The object of the present series of experiments was to test these two possibilities.

METHODS.

The preparation used for testing the first possibility was that described by Rössler [1925]. The frog (*Rana temporaria*) was pithed and the heart removed and placed in Ringer's solution, where it was allowed to beat for a few minutes to wash the blood from it. A cut was then made in the ventricle from apex to base, dividing it into two halves. One half was discarded and the other half, to which the auricles remained attached, was suspended in a bath of Ringer's solution. The apex of the ventricle was attached to a fixed glass tube by means of a loop of silk thread, while a second thread passed through the angle formed by the A.-v. groove and one of the cut edges of the ventricle, and connected this point to a lever. The Ringer's solution had the composition NaCl 0.58 p.c., KCl 0.022 p.c., CaCl_2 0.008 p.c., H_3BO_3 0.062 p.c., CH_3COONa 0.082 p.c., pH 7.5, and was stirred and oxygenated by a stream of oxygen bubbles. Electrodes were arranged so that either auricle or ventricle could be stimulated. Two silver wire electrodes, attached to coils of fine wire so that they were freely movable, were connected to one terminal of the secondary of an induction coil. One of these hooked into the auricular muscle, the other into a small loop in the silk thread connecting the ventricle to the lever. A third electrode, connected to the other terminal of the induction coil, dipped into the Ringer's solution. The points of attachment of the fine electrodes protruded above the surface of the Ringer's solution. Switches were included in the connections of the fine electrodes with the coil, so that either auricle or ventricle could be stimulated at will. Rhythmic shocks were produced by means of a rotary contact breaker in the primary circuit of the induction coil. In order to avoid complication of the results due to stimulation of vagus and sympathetic nerve endings in the heart muscle, atropine and ergotoxine were added to the Ringer's solution in the bath so that the final concentrations were 0.01 and 0.001 p.c. respectively; threshold stimuli were used. Between setting up the preparation and beginning observations 2 hours were allowed for the muscle to come into equilibrium with the Ringer's solution.

A rate of stimulation was then chosen which was slightly higher than the natural auricular rate, and the preparation was driven at this rate from the auricle. A record of the ventricular contractions was taken, and the switches then changed over so that the ventricle was stimulated directly. After a few minutes of this direct stimulation, the preparation was again stimulated from the auricle. Finally, when several such

changes of the point of stimulation had been made, and at the beginning of a period of direct ventricular stimulation, the auricles were cut off at the A.-v. groove. In this way there was no break in the sequence of the ventricular contractions. Fig. 1 shows the record obtained in such an experiment, which is typical of those performed. It is evident that, before cutting away the auricles, the amplitude of the ventricular contractions is not affected by changing the point of stimulation. Removal of the auricles produced a very small, temporary increase in amplitude, which was probably due to the slight unavoidable stretching of the muscle during the cutting. This slight increase had disappeared 1 min.

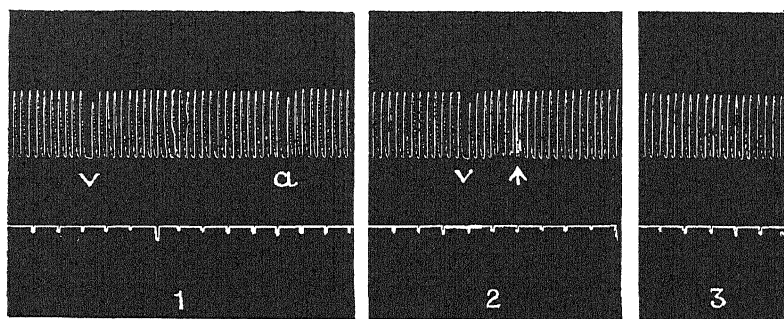


Fig. 1. (1) At beginning of record preparation is driven from auricle. *v* indicates change of point of stimulation to ventricle. *a* return to auricle. (2) $1\frac{1}{2}$ min. later. At \uparrow auricles were cut off. (3) Sample of record 9 min. after end of (2). Stimulation rate 40 per min. throughout. Time marker in this and subsequent figures, 5 sec.

later, and a sample of the tracing taken 9 min. after this shows that there was no permanent change in the amplitude.

It may be argued that artificial stimulation of the auricle will eliminate any natural inhibitory action which the auricle may exert on the ventricle. If this were the case, however, driving from the auricle should produce an increase in the amplitude of the ventricular contractions. Actually a slight decrease is always observed when stimulation is begun, on account of the increase in rate.

It seems likely, from the result of these experiments, that the increase in amplitude of ventricular contractions observed, when the auricles are removed from a heart beating at its natural rhythm, is due to concomitant slowing of the rhythm and experiments with strips of ventricle confirm this. Fig. 2 is taken from an experiment which was begun in the same manner as that described above, namely the auricles were cut off while

the rate was maintained constant by artificial stimulation. One minute after stopping the artificial stimulation of the ventricle a slow idio-ventricular rhythm developed. The amplitude of the first contraction at this natural rhythm was small, and was followed by a short staircase. After this, the amplitude still continued to increase very gradually, until a maximum was reached which was far greater than the amplitude of the contractions at the fast artificial rhythm. Subsequent stimulation at the same rapid rate caused a return to the amplitude of contraction previously observed at this rate. It is plain from this experiment that an increase in amplitude of contraction only occurs when the ventricle is separated from the auricles, and the rhythm is allowed

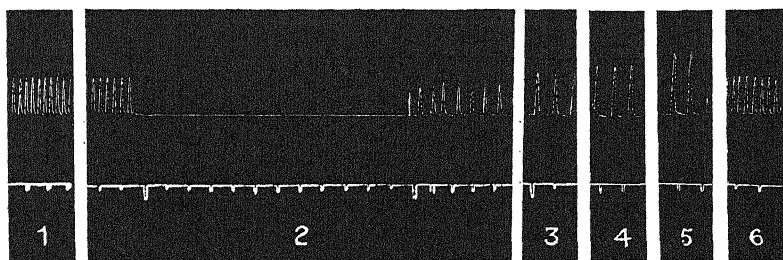


Fig. 2. (1) Preparation driven from auricle at 38 per min. (2) After removal of auricles, driving from ventricle at 38 per min. Driving stopped, and natural rhythm of 18 per min. develops. (3) 20 min. later. Natural rhythm 16 per min. (4) 18 min. after (3), 16 per min. (5) 47 min. after (4), 18 per min. (6) After 40 min. driving at 38 per min.

to slow. There is one point, however, in which this result differs from that obtained by other workers, particularly Rössler [1925]. Whereas in my experiments, of which Fig. 2 is an example, the first contraction at the idio-ventricular rhythm was always smaller than those of the auricular rhythm, in his experiments it was always greater. Moreover, in my experiments the staircase was always present, but in his the staircase was diminished. In this connection I have found that an increase in the calcium chloride content of the Ringer's solution above a concentration of 0.01 p.c. decreases the intensity of the staircase effect, that is, it increases the ratio of the amplitude of the first beat of the staircase to the final amplitude. The Ringer's solution used by Rössler contained 0.02 p.c. CaCl_2 . A series of experiments was therefore performed on strips of ventricle artificially stimulated, using Ringer's solution of the same composition as before, except that the calcium chloride concen-

tration was 0.02 p.c. Stimulation was started at a rate of 36 per minute, and when a constant amplitude had been attained at this rate, stimulation

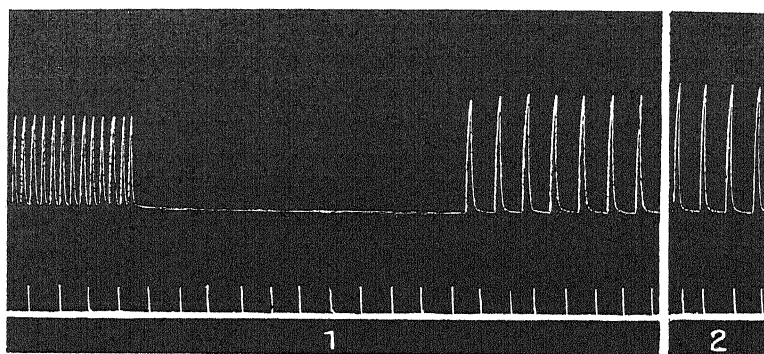


Fig. 3. Isolated strip of *R. temporaria* ventricle. Borate-acetate Ringer. CaCl 0.02 p.c. (1) Stimulation rate at beginning of tracing 36 per min. Pause 1 min. Stimulation resumed at 12 per min. (2) $6\frac{1}{2}$ min. later.

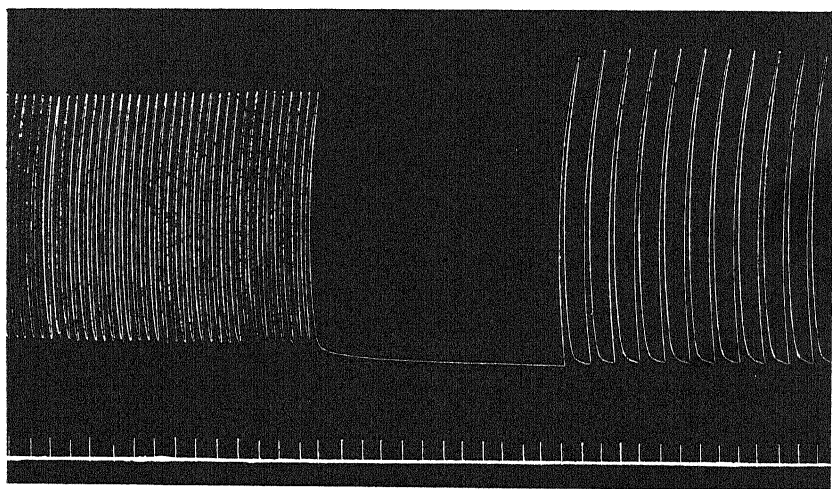


Fig. 4. Isolated strip of *R. esculenta* ventricle. Bicarbonate Ringer. CaCl 0.02 p.c. Stimulation rate at beginning of tracing 27 per min. Pause 1 min. Stimulation resumed at 9 per min.

was stopped for one or more minutes, and was subsequently resumed at a rate of 12 per minute. It was then found that the first beat after the pause was larger than those at the fast rhythm preceding the pause,

whereas with 0.01 p.c. it was smaller. The tracings so obtained (see Fig. 3) resembled those which Rössler obtained by cutting off the auricles. In the heart of *R. temporaria*, therefore, the amplitude of the first beat after a pause is determined by the Ca concentration of the borate-acetate Ringer's solution used. As Rössler's experiments had been carried out on the hearts of *R. esculenta* it seemed desirable to determine whether in this species also the amplitude of the first beat after a pause depended on Ca-ion concentration. It was found, however, that in this species another factor is necessary to produce enlargement of the first beat, namely the Ringer's solution must be buffered with bicarbonate instead of borate and acetate. In a strip of *R. esculenta* ventricle immersed in a borate-acetate Ringer's solution with CaCl_2 concentration of 0.02 p.c. the first beat after a pause is always smaller than those at a fast rhythm which precede the pause. If, however, the Ringer's solution contains 0.02 p.c. CaCl_2 and is buffered with bicarbonate (as was the solution used by Rössler) the enlargement of the first beat is always observed. The reason for this difference has not as yet been investigated. Fig. 4 shows the record of an experiment. It will be noticed that the contractions following the pause are greater, not only on account of the fact that the lever rises to a greater height, but also on account of a fall of the base line. Such a fall of base line has been observed by others [Szent-Györgyi, 1920; Regelsberger, 1922], in the record of ventricular contractions when the auricles are removed, and has been regarded by them as proof that the auricles maintain the ventricular muscle in a state of tone. It is evident from the present experiments that this so-called diminution of tone is not necessarily due to the removal of the influence of the auricles.

SUMMARY.

1. The increase in amplitude of contraction of the frog's ventricle observed when the auricles are cut off is entirely due to the slowing of the rhythm which also occurs. There is no change in ventricular amplitude when the auricles are cut off while the rhythm is maintained artificially constant.
2. An increase of amplitude, identical with that observed when the auricles are removed, can be produced by slowing the rhythm at which an isolated ventricular strip is driven.

This work was begun in the Physiological Laboratory, Cambridge, and concluded in the Department of Physiology of the University of

Birmingham. I wish to express my thanks to Prof. Barcroft and Prof. de Burgh Daly for permission to carry out my experiments in their respective departments.

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THE REACTIVITY AND ACTIVITY OF THE RABBIT'S
UTERUS DURING PREGNANCY, PARTURITION
AND THE PUERPERIUM.

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WHEN suspended *in vitro* in oxygenated physiological solution the uterus of the oestrous rabbit is capable of reacting to an extract of the posterior lobe of the pituitary, "pituitrin," and both the oxytocic and pressor fractions can cause contraction of the muscle. During pseudo-pregnancy and the first half of pregnancy, the reactivity of the muscle to these preparations is abolished [Knaus, 1927, 1930]; indeed pituitrin may even cause inhibition of the spontaneous activity and relaxation of the tone, this being chiefly due to vaso-pressin [Robson, 1932].

Knaus [1930], who also investigated the reactivity during the later stages of pregnancy, obtained varying results; sometimes the addition of "pituitrin" caused contraction, while in other experiments no effect resulted. Only at the very end of gestation could contraction of the uterus be constantly obtained with it. He deduced from these experiments that the reaction of the uterus at term to the influence of the posterior lobe initiated parturition. As the hypothesis that oxytocin plays an important part in parturition has been accepted by a number of investigators, it seemed desirable to determine more accurately the reactivity of the uterus during the later stages of gestation and to obtain quantitative data on the effect of standard preparations from the posterior lobe on the organ. At the same time certain of the physiological changes in the organ during the puerperium were also determined.

METHODS.

The experiments were performed on mature female rabbits of various breeds weighing about 2 kg. each. Mating was observed and the state of the uterus then investigated at fixed stages of gestation. The animals

were all operated on under ether anæsthesia, and strips of muscle removed. In some experiments the part of the uterus corresponding to a whole embryo was completely removed and the uterine strips subsequently dissected from this, while in others the muscle was removed directly from the uterus, the corresponding embryo and placenta also removed, and the incision sewn up. As a rule these operations caused little interference with the other embryos and it was possible to remove several strips in one and the same animal at various succeeding stages of gestation.

The muscle strips were suspended in oxygenated Ringer-Locke solution in 100 c.c. containers and records taken on smoked drums. The standard preparations from the posterior lobe which were used were "pitocin" and "pitressin," kindly supplied by Messrs Parke, Davis and Co.

RESULTS.

In all experiments an attempt was made to determine the minimal amount of drug added to the solution which caused a motor effect on the muscle. In the majority of experiments the response obtained included a rise in the base line. In some experiments when the effect of small doses was being investigated, however, the chief effect was an increase in the height of the rhythmic contractions, with little or even no effect on the base line. In a few cases the chief action was to increase the rate of the rhythmic contractions. All experiments were performed in duplicate and the reactivity given for any one experiment and expressed as units added to a 100 c.c. of solution is always that of the strip with the higher reactivity. The conditions were, within very narrow limits, exactly alike for the two strips and, as a rule, similar results were obtained with them. In some cases fairly marked differences of reactivity were observed between two strips removed at the same time from one animal, suggesting that the whole uterus did not possess a uniform reactivity at a definite stage of pregnancy. All the experiments were carried out over fairly long periods extending sometimes up to 10 hours. More consistent results were obtained by this method, since it was found in some cases that the muscle did not exhibit its maximal reactivity until it had been in the bath for one or more hours. The figures given in all cases represent the maximal reactivity observed in any one experiment.

In the first half of pregnancy, as had been previously shown by Knaus, large doses of pituitrin (up to 5 units added to 100 c.c. of

solution) were unable to cause any motor effects. This was followed by a stage in which comparatively large amounts of pituitrin caused contraction. On investigation at this stage, it was found that oxytocin did not cause any effect, while vaso-pressin brought about contraction of the muscle. This is illustrated in Fig. 1. The duration of this anomalous

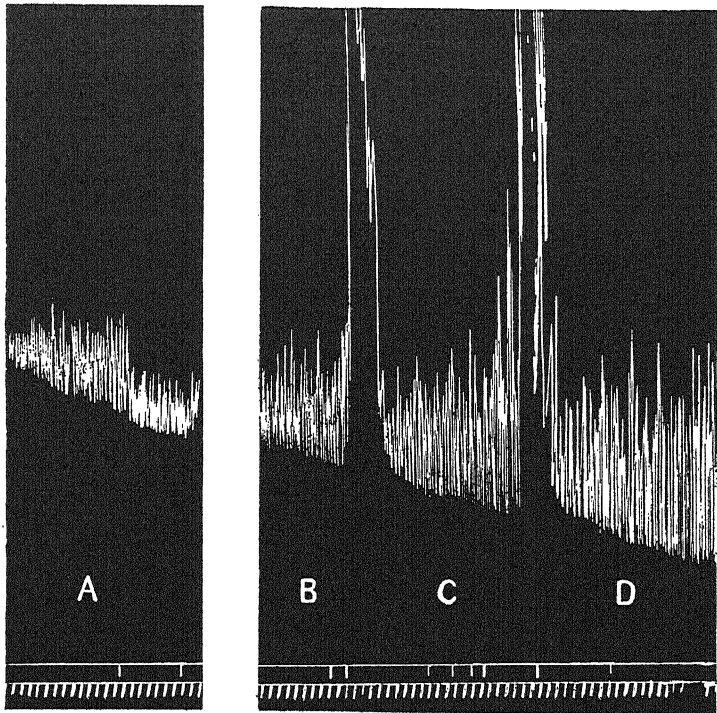


Fig. 1. Reactions of uterine strip removed from animal (Ra 338) 18 days pregnant. A, 2 units of pitocin added to bath. No effect. B, 2 units of pitressin added to bath. C, 0.1, 0.1, 0.1 and 0.2 unit of pitressin successively added to bath. D, 1 unit of pitocin added to bath. Solution changed each time. Time intervals=1 min.

condition varied in the different animals. In the series investigated it was actually observed on the 18th, 23rd and 27th days of pregnancy (see Table I).

In some animals, however, the reactivity to oxytocin returns before the last few days of pregnancy. Thus in one case (Ra 336) a high degree of reactivity was already observed on the 21st day of pregnancy; several live embryos were at that time present in the uterus. In another animal

(Ra 334) an extremely high reactivity was observed on the 25th day of pregnancy. This animal had been operated on twice previously, and at the laparotomy on the 25th day only one apparently live embryo was left. The strips of muscle used for the investigation were removed from a part of the uterus containing an absorbing embryo. Five days later a further laparotomy was performed on this animal and no live embryos were found in the uterus. In other animals (338, 342, 343, Table I) the reactivity to oxytocin, even up to the 28th day of gestation, was comparatively small and doses of the drug of 0.1 unit or more had to be added to the 100 c.c. of solution before any appreciable motor effect was obtained.

TABLE I. To show the reactivity of the uterus at various stages of pregnancy and at parturition. Where the exact dose has not been determined, the maximal or minimal ineffective dose that has been used is stated.

Exp. No.	Day of pregnancy	Effective dose of drug				Remarks
		Pituitrin units	Pitocin units	Pitressin units	Histamine mg.	
Ra 334	18	—	>3	<2	—	—
Ra 336	18	5	>2	>2	—	—
Ra 338	18	—	>2	0.5	<0.05	—
Ra 335	19	—	>2	>2	—	—
Ra 346	19	1	>1	—	0.002	—
Ra 334	20	—	<2	<1	—	—
Ra 336	21	—	0.01	0.2	0.002	—
Ra 335	23	—	>2	0.5	—	—
Ra 334	25	—	0.003	0.02	—	{ Only 1 full-sized embryo remained. Strips removed from part of uterus containing absorbing embryo
Ra 342	25	—	>2	>1	0.006	
Ra 343	25	—	0.2	—	0.002	—
Ra 342	27	1	>0.5	0.5	0.004	—
Ra 338	27	—	0.3	—	—	Parturition on the 30th day
Ra 340	28	—	0.004	—	0.005	After birth of 1 foetus
Ra 342	30	—	0.01	—	—	After birth of 3 foetuses
Ra 343	31	—	0.002	—	0.002	After birth of 5 foetuses
Ra 389	31	—	0.003	—	—	On morning after parturition
Ra 392	32	—	0.02	—	—	More than 12 hours after parturition
Ra 339	33	—	0.001	—	0.002	On morning after parturition

The reactivity of the uterus at, or shortly after, parturition was investigated in six animals and under the following conditions:

Ra 340. Parturition 28 days after mating. The bursting of the membranes was observed and one foetus born precipitately. The rabbit was

immediately operated on and strips of muscle dissected from the part of the uterus which had contracted on the placenta, which had not yet been expelled; three other foetuses were present in the uterus.

Ra 342. Parturition 30 days after mating. The birth of three foetuses was observed. The animal was then immediately operated on and muscular strips were removed. The uterus contained no live foetuses, but one absorbing embryo.

Ra 343. Parturition 31 days after mating. The birth of a litter of five was observed. The animal was then immediately operated on and muscular strips were removed. No further foetuses were left in the uterus.

Ra 389. Parturition occurred during the night of the 31st-32nd day after mating; 5 foetuses were born. The next morning strips were removed at operation. No foetus was left in the uterus.

Ra 392. Parturition occurred during the night of the 31st-32nd day after mating; seven foetuses were born. On the afternoon of the next day the animal was operated on and muscular strips removed. No foetus was left in the uterus.

Ra 339. Parturition occurred during the night of the 32nd-33rd day; nine foetuses were born. The next morning strips were removed at operation. No foetus was left in the uterus.

As will be seen in Table I, the reactivity to oxytocin of the strips removed at or shortly following parturition is very high. The uterus which showed the least reactivity (*Ra 342*) gave a contraction when 0.01 unit of pitocin was added to a 100 c.c. of solution, while in the case of *Ra 339* when parturition occurred between 32-33 days after mating, the addition of 0.001 unit of pitocin was sufficient to bring about a motor effect (Fig. 2). In one case (*Ra 392*) the strips were removed more than 12 hours after parturition and the reactivity was slightly less, 0.02 unit being necessary to bring about contraction of the muscle.

Histamine. The reactivity to histamine of a number of the strips at various stages of pregnancy was also investigated, the minimal dose necessary to bring about a motor effect being in all cases determined.

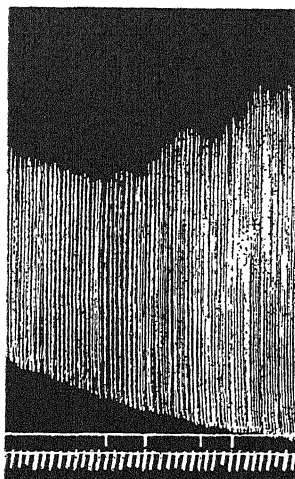


Fig. 2. Reaction of uterine strip removed from animal (*Ra 339*) on morning after parturition. Successive additions of 0.0005, 0.0005, 0.001 and 0.002 unit of pitocin. Time intervals = 1 min.

It was found that during the stage investigated (19th day to full term) the reactivity to histamine did not show any increase. The doses necessary to cause contraction were in all cases relatively small, the effect being produced by the drug in concentration of 1 in 50,000,000 to 1 in 16,000,000 (Table I).

The reactivity during the puerperium. The morphological and histological changes in the uterus have been described by Hammond [1925], who showed that the puerperal changes were influenced by lactation. The involution process proper which occupies about a week, is similar both in suckling and non-suckling animals, but the subsequent atrophy is more marked in suckling does. The reactivity changes were therefore studied in both suckling and non-suckling animals and the results were collected in Table II (in which, for comparison, the results for parturition

TABLE II. To show the reactivity of the uterus at parturition and at various stages of the puerperium.

Exp. No.	Days of puerperium	Minimal effective dose of drug			Remarks
		Pitocin units	Pitressin unit	Histamine mg.	
Ra 340	0	0.004	—	0.005	—
Ra 342	0	0.01	—	—	—
Ra 343	0	0.002	—	0.002	—
Ra 389	<1	0.003	—	—	—
Ra 339	<1	0.001	—	0.002	—
Ra 392	1	0.02	—	—	—
Ra 342	2	0.2	—	—	No suckling
Ra 343	3	0.002	—	0.01	„
Ra 392	5	>0.3	—	—	„
Ra 371	5	0.5	—	—	Suckling
Ra 352	6	0.3	—	0.04	„
Ra 368	7	0.1	—	>0.08	No suckling
Ra 389	7	0.5	—	—	„
Ra 355	7	0.5	—	0.02	Suckling
Ra 364	9	0.5	—	—	No suckling
Ra 367	12	0.5	—	—	Suckling
Ra 355	13	0.004	—	0.01	„
Ra 352	13	0.01	—	0.01	„
Ra 389	17	0.02	—	—	No suckling
Ra 371	17	>0.3	—	>0.07	Suckling
Ra 368	18	0.03	—	0.03	No suckling
Ra 364	23	0.03	—	0.01	„
Ra 345	23	0.5	—	>0.2	Suckling
Ra 352	27	0.1	—	0.01	„
Ra 355	32	>0.5	0.5	0.01	„
Ra 389	32	0.1	—	—	No suckling
Ra 364	35	0.1	—	>0.07	„

are also included). As the effects were practically similar in both sets of animals and appeared to be independent of suckling, they will be described together. There is a rapid fall in reactivity to oxytocin during the first week following labour. Thus in the majority of animals the dose

of oxytocin necessary to bring about a motor effect becomes, within a week, 100 times greater than that effective at parturition. The rate of decrease of reactivity varied in different animals. For example, in one case (Ra 342) the effective dose of oxytocin increased by 20 times between parturition and the 2nd day of puerperium, while in another animal (Ra 343) there was no apparent change during the first 3 days of the puerperium. For the week after that the reactivity was low in all animals investigated (Table II).

It does not, however, permanently remain at the low level but increases again later on in the puerperium and may become quite high, though as a rule not so high as at parturition: for instance, as early as the 13th day (Ra 352 and 355), and as late as the 23rd day after parturition. In a number of suckling animals (Ra 345, 352 and 355) in which extreme atrophy of the uterus occurs, the uterine reactivity was examined on the 53rd, 60th and 62nd day after mating, and was found to have again decreased, so that doses of oxytocin of the order of 0.1 unit were necessary to cause contraction. A similar result was obtained with two non-suckling animals (Ra 389 and 364) observed 63 and 65 days after mating.

As regards the reactivity of the puerperal uterus to histamine, it was found in general that the doses of the drug necessary to cause a motor effect were rather larger than at parturition, though the increase was not nearly so marked (Table II) as in the case of oxytocin. In a few experiments, however, the reactivity to histamine was considerably decreased in the puerperal uterus (Ra 368, 371, 345, 364).

Variations in the spontaneous rhythmic activity were studied similarly at the different stages of pregnancy and of the puerperium, and the results are tabulated in Table III. The notation for different degrees of activity is arbitrary, but they can be estimated within fairly accurate limits. When completely absent or very small the minus sign has been entered in column 4 (Table III), while increasing heights and rates of contractions are represented by an increasing number of plus signs. Pieces of muscle as nearly as possible of the same length were used. In the great majority of cases the two strips used in any one experiment gave very similar results; in a few cases, where the results were widely divergent, they are both given in the table. It must also be mentioned that the strips may only attain their maximal rhythmic activity after being in the bath for some considerable time; this maximal activity is then usually maintained for a long period and is actually represented in Table III.

TABLE III. Relating spontaneous activity and reactivity to oxytocin at various stages of pregnancy and the puerperium.

Exp. No.	Days after mating	Minimal effective dose of pitocin units	Spontaneous activity	Remarks
Ra 339	33	0.001	- - + +	On morning after parturition
Ra 343	31	0.002	+ + + +	Immediately after parturition
Ra 343	34	0.002	-	—
Ra 339	31	0.003	-	On morning after parturition
Ra 334	25	0.003	+ + +	—
Ra 340	28	0.004	- + + + +	After birth of 1 foetus
Ra 355	43	0.004	+	—
Ra 342	30	0.01	+ + + +	Immediately after parturition
Ra 336	21	0.01	+	—
Ra 352	46	0.01	+ +	—
Ra 392	32	0.02	-	More than 12 hours after parturition
Ra 364	53	0.03	-	—
Ra 368	48	0.03	+	—
Ra 343	28	0.1	+ + +	—
Ra 368	37	0.1	+ + +	—
Ra 352	60	0.1	+ + +	—
Ra 364	65	0.1	+	—
Ra 342	32	0.2	+ + + +	—
Ra 343	25	0.2	+ + +	—
Ra 338	27	0.3	+ + +	—
Ra 352	39	0.3	+ +	—
Ra 389	38	0.5	+ + +	—
Ra 355	37	0.5	+ +	—
Ra 364	39	0.5	+ +	—
Ra 367	42	0.5	+	—
Ra 345	53	0.5	+	—
Ra 371	35	0.5	+ +	—
Ra 392	37	>0.3	+ +	—
Ra 371	47	>0.3	+	—
Ra 342	27	>0.5	+ - + + +	—
Ra 346	19	>1.0	+	—
Ra 342	25	>2.0	+	—
Ra 335	23	>2.0	+	—
Ra 335	19	>2.0	+	—
Ra 338	18	>2.0	+	—
Ra 336	18	>2.0	+ + + +	—
Ra 334	18	>3.0	+ +	—

An analysis of this table shows that there exists no obvious direct relation between the rhythmic activity of the muscle and its reactivity to oxytocin. They vary independently. Thus, for example, muscle strips removed from animal Ra 343 3 days after parturition were made to contract by the addition of 0.002 unit of oxytocin to the bath, although they showed no spontaneous activity whatsoever (Fig. 3), while strips removed from Ra 336 on the 18th day of gestation showed a very high degree of rhythmic activity, yet were unaffected by the addition of 2 units of oxytocin to the bath. Furthermore, strips removed from one animal at the same time may show widely different rhythmic activities,

although the reactivity to oxytocin is substantially the same. Thus two strips of muscle were removed from Ra 339 on the morning after parturition. One of these showed only very slight, the other quite marked rhythmic activity; in both cases 0.001 unit of oxytocin caused a motor effect; the same was observed in Ra 340 from which two strips were removed immediately after the birth of one foetus; one strip showed marked rhythmic activity and was caused to contract by the addition

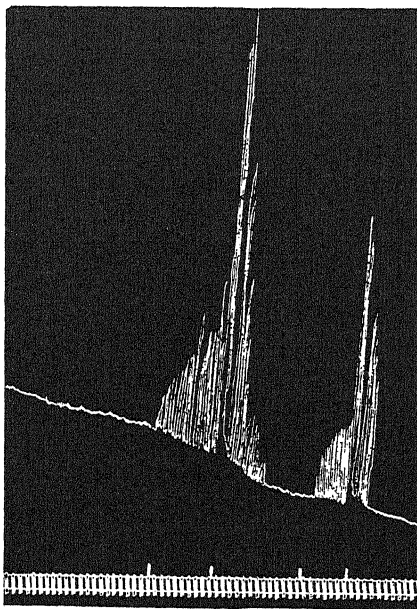


Fig. 3. Reaction of uterine strip removed from animal (Ra 343) 3 days after parturition. First signal: 0.005 unit of pitocin added to bath. Second signal: solution changed. Third signal: 0.002 unit of pitocin added to bath. Time intervals = 1 min.

of 0.004 unit of oxytocin to the bath, while a dose of 0.007 unit caused a contraction of the other strip which exhibited only very slight contractions.

Examination of the figures also shows that there is no obvious relation between the spontaneous activity exhibited *in vitro* and the period of pregnancy and the puerperium (within the limits investigated) at which the strips are removed. A possible exception to this general statement is that the highest rhythmic activities (++++), with one exception (Ra 336), all observed in strips removed during or shortly following parturition.

DISCUSSION.

The experiments show that the reactivity of the rabbit's uterine muscle to the posterior lobe hormones during gestation follows a fairly definite course, and may be divided into the following consecutive stages:

(1) The first half of pregnancy (*i.e.* the first 16 days) marked, as already shown by Knaus [1930], by a complete absence of reactivity to large doses of oxytocin and vaso-pressin.

(2) A stage in which the oxytocic hormone has no effect upon the muscle while the pressor hormone causes it to contract. The doses of vaso-pressin necessary to bring about contraction are, however, fairly large and the reaction may not have much physiological significance.

(3) The later part of gestation, during which the reaction to oxytocin always returns, though the exact time at which a given dose of the drug causes contraction varies in the different animals. Once established, the reactivity increases during the later phases of pregnancy.

(4) Parturition during and immediately following which the reactivity to oxytocin is always high, although the actual dose necessary to cause contraction varies in the different animals. These variations may, to some extent, be due to small differences in the experimental procedure; but it seems nevertheless likely that they are outside the limits of experimental error, and that the reactivities at parturition in different uteri or even in different parts of the same uterus are not constant.

However, the experiments support the general conclusion that the reactivity of the uterus gradually increases during the later phases of gestation and attains a maximum at parturition. Moreover, this increase does not affect the general reactivity of the muscle to all stimuli, as is evidenced by the results obtained with histamine.

During the week following parturition involution of the organ is completed [Hammond, 1925], and at the same time its reactivity to oxytocin undergoes a very rapid decrease. The rate of decrease varies in the different animals, but the dose necessary to cause contraction is in all cases high by the 5th day of puerperium and remains so for at least a week. The changes in reactivity are independent of whether the animal is suckling or not. An attempt is made in Fig. 4 to represent graphically the changes in the reactivity to oxytocin in the rabbit's uterus at the various stages of pregnancy and the puerperium. It must be remembered that fairly wide variations occur in the different animals and that the figures used represent only the general course of events as deduced from the observations on the whole series.

The examination of the spontaneous rhythmic activity at the various stages of pregnancy and of the puerperium leads to two conclusions. In the first place it seems to be definitely established that there is no relation between the reactivity of uterine muscle to oxytocin and the rhythmic activity exhibited *in vitro*. Secondly, the various stages of the cycle investigated are not associated with any characteristic type of spontaneous activity, except that very marked rhythmic contractions may be observed during parturition. It has been shown by Reynolds

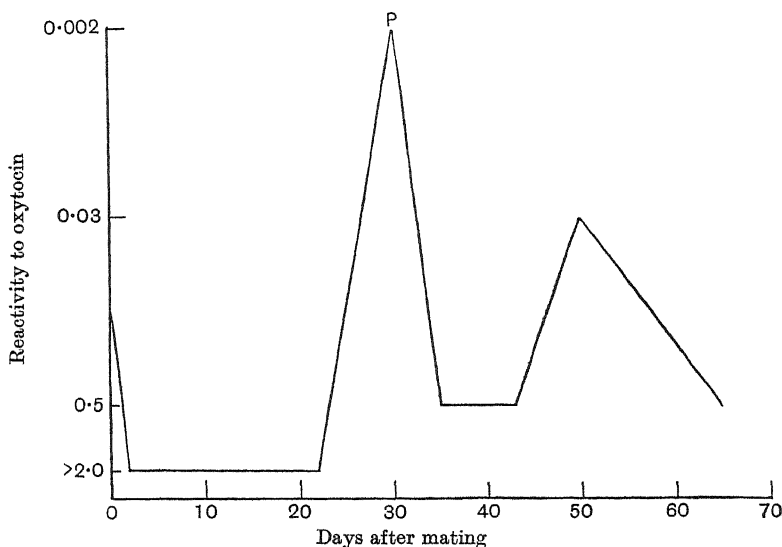


Fig. 4. Schematic representation of the uterine reactivity to oxytocin (expressed as the minimal number of units added to 100 c.c. of Ringer-Locke which causes a motor effect) at various stages of pregnancy and the puerperium. P=parturition.

[1932] and Reynolds and Allen [1932] that the rhythmic activity of the rabbit's uterus, when observed *in vivo*, is influenced by definite hormonal factors, including oestrin and progesterin. The findings with the isolated strips suggest that changes in the rhythmic contractility of uterine muscle due to ovarian hormones are not of such a kind as to persist when the muscle is removed from the body and observed *in vitro*; it appears more likely that the alterations in rhythmic activity observed by Reynolds and Allen are dependent on the quantities of hormone actually present in the muscle during the investigation. Moreover, it must also be concluded that the hormonal factors influencing spon-

taneous contractions *in vitro* do not necessarily exert a similar effect on the reactivity of the uterine muscle to oxytocin.

The secretory activity of the corpus luteum appears to be an important, if not the determining factor in causing the lack of uterine reactivity during the first half of gestation [Knaus, 1930], and it seems indeed quite possible that a specific hormone acting on the uterine muscle is produced [Robson and Illingworth, 1931; de Fremery, Lucks and Tausk, 1932]. It has been suggested by Knaus that the reactivity to oxytocin at the end of gestation is the factor which determines the onset of parturition; and an examination of the data given above tends on the whole to support this hypothesis, for the later stages of gestation are marked by an increase in reactivity to oxytocin, and parturition is always marked by a very high reactivity to this hormone. At the same time it must not be forgotten that in two cases strips of muscle removed several days before parturition showed a high degree of reactivity to oxytocin, in one of them as early as the 21st day of gestation. It is, however, possible that these results were due partly to the previous operative interferences and to experimental error and that they do not necessarily disprove the general theory.

It now remains to discuss whether the increase in reactivity characteristic of the later phases of pregnancy is merely the result of a decrease in the luteal control, and whether the very high reactivity characteristic at parturition simply follows the complete withdrawal of the luteal secretion. Certain facts make the acceptance of the view rather difficult. In the first place it has been shown by Courrier and Kehl [1929], and later by Knaus [1930] that the progestational reaction due to the corpus luteum hormone is no longer present for over a week before the end of gestation. This suggests that the corpus luteum does not continue to exert its activity up to the end of pregnancy. More important still, however, are the changes in reactivity observed during the early stages of the puerperium, when the reactivity to oxytocin of the uterine muscle rapidly decreases. Such findings would hardly be expected if the high reactivity at parturition was merely due to the withdrawal of the inhibitory luteal secretion, and they suggest the possibility that some active factor is concerned.

SUMMARY.

The reactivity to oxytocin and vaso-pressin of strips of rabbit's uterus removed at various stages of pregnancy, parturition and the puerperium has been quantitatively determined and definite stages have

been established. The response to histamine does not show similar variations.

The spontaneous rhythmic activity at the various stages has also been determined and it is shown that no stage during the period investigated is characterized by any definite type of rhythmic activity; a possible exception to this observation is provided by the fact that a very high degree of rhythmic activity has several times been observed at parturition.

There appears to be no relation between the spontaneous activity of the uterine strips and their reactivity to oxytocin.

It is emphasized that parturition marks the apex in the reactivity curve of the uterus to oxytocin. The relation of the findings to the hormonal factors operating on the uterine muscle is discussed, and it is suggested that the high reactivity observed at parturition may not be due merely to the withdrawal of the action of the corpus luteum.

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THEORIES OF MUSCULAR CONTRACTION.

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THE work of Fletcher [1899] on CO_2 production and of Fletcher and Hopkins [1907] on lactic acid production in muscle sufficed to dispose of the "inogen" theory of muscular contraction in its usual form. They showed that the supposed breakdown products of the explosion of "inogen" were not formed at the time and in the manner predicted by the theory. Their work did not dispose of the assumption which underlay the erroneous chemical theory and which has always been accepted; namely, that the resting muscle contains some system of high potential energy which breaks down on stimulation to liberate energy available for mechanical work. A further assumption has generally been made that the stored energy was chemical potential energy, as in the "inogen" theory. The change of view was merely to substitute a different kind of chemical mechanism. The supposed sequence of events in a muscle was (1) stimulation, (2) an exothermic chemical reaction, producing (3) contraction and relaxation, then (4) recovery reactions of greater free energy than (2) restoring the chemical system destroyed. For brevity this will be called "The Chemical Theory." There is another alternative which has as yet received little attention, that the stored potential energy might be mechanical or electrical, at any rate not chemical in the sense used above, and that all known chemical reactions might belong to the recovery process by which the store was replenished. This may be called "The Physical Theory." I have recently advocated such a view [Ritchie, 1932], but in a form I now realize to be seriously defective.

As long as it was possible to assume that the primary chemical change was the formation of lactic acid from carbohydrate, it was plausible to treat this as the causal process producing contraction. This reaction appeared to explain the two phases of activity, contraction and relaxation, and liberation of acid appeared to be the kind of process that might reasonably produce in a colloidal system the kind of mechanical effects observed.

However, the observations of Lundsgaard [1930, *a*, *b*; 1931 *a*, *b*] have shown that a muscle can contract normally while no lactic acid appears. Mawson [1932] and Smith and Visscher [1932] find that the non-appearance of lactic acid cannot be explained away as a result of the reversal of the reaction: carbohydrate \rightarrow lactic acid. Finally it has been shown that when lactic acid does appear it is formed mainly, if not entirely, after contraction [Lehnartz, 1931; Meyerhof, 1931; Lundsgaard, 1931 *b*]. If the chemical theory is to be saved, phosphagen breakdown must hold the place so long theoretically occupied by lactic acid production. But the general plausibility of the theory is now so much less, that it is essential to consider the alternative one that phosphagen breakdown is simply the first and quickest of a series of reactions forming the recovery process.

The question at issue between the chemical and physical theories could be settled if the time relations of phosphagen breakdown were accurately known. (1) In accordance with the chemical theory, the phosphagen breakdown, or better, the accelerated phosphagen breakdown, accompanying activity should begin before or as soon as contraction begins, reach its maximum before or not later than the height of contraction and sink to the resting value at least as fast as the muscle relaxes. Or else (2) in accordance with the physical theory, the absolute refractory period should mark the time of liberation of energy from the preformed store. The recovery reactions, headed by phosphagen breakdown, should begin to come into play towards the end of the absolute refractory period, and the relative refractory period should mark the time of maximum rate of phosphagen breakdown. But clearly on this view there is a steady phosphagen breakdown (balanced by steady re-synthesis) during rest in order to maintain the store of potential energy, and this is simply increased after excitation to replenish the store. The increase will occur as quickly as the nature and conditions of the chemical reaction permit. A direct chemical attack on this problem seems out of the question at the present time, but an indirect attack may be of some use.

If the physical theory is correct any variation in conditions which slows or quickens phosphagen breakdown should alter the duration of the refractory period in the same sense. The two theories are not simply contradictory, for this one reaction may have both supposed functions or neither; so that it is necessary to test the chemical theory independently. This is not easy to do, but there is one obvious experiment which may have some bearing on the question; that is to see whether a

change of conditions to slow or quicken phosphagen breakdown can be correlated with the rate of development of contraction or more simply with the latent period. As it is ordinarily measured the latent period is an artifact [Roos, 1932]. Part of it is due to the taking up of slack in muscle and recording apparatus and to the inertia of the apparatus, but with any given muscle and set up of apparatus these factors will be constant. Another part of the latent period is due to muscle viscosity and will vary if it varies. However, as long as all these factors are constant any change in speed of a causal reaction might be expected to change the apparent latent period in the same sense. That is to say the time taken for the contractile process to reach such a value as to overcome slack, viscosity and inertia and produce a visible movement should vary with the speed of phosphagen breakdown if that is the causal reaction.

According to Meyerhof and Lohmann [1928 *a*] the reversible reaction, $\text{phosphagen} \rightleftharpoons \text{creatine} + \text{phosphate}$ is catalysed *in vitro* by muscle enzymes. At reactions between *pH* 6 and 7 the equilibrium is shifted most to the right, at reactions more alkaline than *pH* 8.5 it is almost completely to the left. Gerard and Tupikov [1931] find that in intact tissues CO_2 concentrations of more than 5 p.c. greatly accelerate phosphagen breakdown, so that similar relations evidently hold in life. It may be assumed that at reactions between *pH* 8 and 9 breakdown will occur relatively slowly, at reactions between *pH* 6 and 7 much faster.

When the phosphagen store of a muscle cannot be renewed as in a muscle poisoned with iodoacetate (I.A.A.) and in nitrogen, it is likely that the breakdown will go slower as the store becomes exhausted. It is possible on the other hand that the rate of breakdown might be controlled by the amount of some catalyst and be largely independent of phosphagen concentration; but even so some variation is probable. The methods of altering the rate of phosphagen breakdown will therefore be (1) change of *pH*, (2) exhaustion of the store, and the effects to be looked for (*a*) change of refractory period, (*b*) change of latent period. The effect of changing the *pH* on the refractory period has already been investigated by Adrian [1920] and the experiments to be recorded merely confirm some of his results under rather different conditions.

METHODS.

The best material for such experiments is the ventricle of the frog's heart, particularly now that the Edinburgh workers have elucidated its metabolism [Clark, Gaddie and Stewart, 1931, 1932; Clark, Eggleton and Eggleton, 1932]. Normally the heart works aerobically

and does not depend to any great extent on carbohydrate oxidation or on the glycogen-lactic acid cycle. The heart treated with iodoacetic acid is practically normal and beats well for hours by resynthesizing its phosphagen by means of non-carbohydrate oxidations. Deprived of oxygen it soon stops from depletion of the phosphagen store, but recovery takes place on readmission of oxygen. For practical purposes the I.A.A. heart may be considered as working on two reactions, (1) phosphagen breakdown, (2) phosphagen synthesis by means of oxidations (neglecting the small anaerobic contribution that may be made by breakdown of adenylypyrophosphate). By cutting off or admitting oxygen (2) can be stopped or started at will.

Other advantages of heart muscle are its slow working; the long refractory period, which is easily measured and precludes summation of contractions; the all or none character of the beat which eliminates complications due to varying numbers of fibres in action at different times. A disadvantage of the heart is that the muscle is very extensible and develops small tensions so that isometric conditions are practically unattainable.

A very simple technique sufficed for the present purpose. A ring of ventricular muscle from a frog's heart was obtained by cutting with scissors parallel to the auricular-ventricular junction and then making another parallel cut nearer the apex. Two silver wires were passed through and bent round to hold the ring. The tissue was suspended in a narrow glass tube. One wire passed through the cork in the bottom, the other was attached to a very light isotonic lever. The wires also served as stimulating electrodes. The tube was filled with about 1 c.c. of a Ringer's solution containing no phosphate but 0.0055 *M* NaHCO_3 and about 1/25,000 sodium iodoacetate. Oxygen, nitrogen, or oxygen and carbon dioxide mixtures were bubbled through the Ringer containing the muscle. The tube containing the muscle was in most cases surrounded with ice during an experiment.

When oxygen or nitrogen was passing the *pH* of the solution was 8.4 to 8.6 at room temperature. A mixture containing 33 p.c. CO_2 produced irreversible stoppage, but below 20 p.c. recovery could be obtained. The concentrations of 5–20 p.c., which were used, gave a range of *pH* 6.8–6.1 at 0° C., calculated from the Henderson-Hasselbach equation using Kendall's [1916] data for 0° C. It may be assumed that the tissue will be rapidly equilibrated to CO_2 , and on the analogy of the red blood corpuscle the internal *pH* will be somewhere about 0.2 less than the external fluid.

The muscle was stimulated with induction shocks through a rotating contact breaker. For moderate rates of stimulation break shocks only were sent in, but for the most rapid rates it was found better to use make and break shocks with the make below the threshold, in order to avoid a rise in threshold from polarization. Frequently there were spontaneous beats when the muscle was first set up, but these usually died out after 10 mins. or so. If they persisted the preparation was rejected. In many cases a muscle that had been brought to a standstill three or four times in the interval was beating well at the end of 4 hours.

The preparations tended to be variable. The viscosity as measured by latent period and rate of contraction and relaxation was generally high at first and tended to fall later. The refractory period was seldom constant for long and tended to become shorter during a prolonged period of normal beating in oxygen. The threshold for stimulation did not vary very much, but there was a tendency for lower thresholds on recovery in oxygen after CO_2 .

Changes in refractory period were determined by stimulating with shocks only slightly above the threshold sent in at a constant rate more rapid than the tissue could follow. The muscle will then break into a faster or slower rhythm of response as the refractory period changes. Where, as in the case of acid conditions, the effect is transient, this method is more suitable than the one of interpolating extra shocks at various times in a slow series of regular stimuli, because if the change is big enough to be detected at all it will not be missed whenever it occurs.

Determinations of latent period were made with a moderate rate of stimulation the heart could easily follow. It might be expected that small contractions would show an apparent longer latent period than large ones. But it was evident that this source of error was negligible (see Table I).

EXPERIMENTAL RESULTS.

A few general observations may be recorded before dealing with the special points at issue.

Effect of nitrogen on I.A.A. muscle. On turning from oxygen to nitrogen there is no visible effect for a time, presumably while the residual oxygen is used up, then the contractions rapidly diminish. The threshold is raised and when the contractions are small they are not always all or none, evidently because conduction has failed. However, a movement of 2 cm. of the secondary coil is enough to change a minimal to a maximal stimulus. Complete recovery in oxygen to give full-sized

contractions is generally slow, but partial recovery from complete inexcitability is quite rapid.

In many cases the heart comes to a standstill in nitrogen completely relaxed and afterwards it may or may not pass into rigor. If it has been worked for a short time only there may be no rigor. After long working rigor usually occurs and may begin while the tissue is still responding (see Fig. 1 B). Incipient rigor can be reversed in oxygen. The suggestion, based on the observations on skeletal muscle that failure of the I.A.A. muscle to respond is failure to relax, is evidently wrong [Ritchie, 1932].

Effect of carbon dioxide. When oxygen containing more than 5 p.c. CO_2 is passed into the I.A.A. heart with continual stimulation the onset of the effect is seen in less than 10 min., but the heart is only gradually brought to a standstill. The effect is very slowly reversed by bubbling oxygen, evidently because of the slowness of evolution of CO_2 . The contractions in CO_2 get slower as they get smaller. The slowing is evidently due to increased viscosity and probably some of the diminution of an isotonic response is due to viscosity also; but this does not explain the total extinction of response. Increase in acidity is known to slow the oxidation processes [Hartree and Hill, 1924], and Mawson [1932] finds that more than 5 p.c. of CO_2 slows down the oxidative resynthesis of phosphagen. The store in heart muscle is very small in any case, only enough for a few full-sized beats [Clark, Eggleton and Eggleton, 1932], so that the final extinction of the response will be due to exhaustion of the phosphagen store by slowing of the resynthesis. The threshold for stimulation is not raised by CO_2 and may even be lowered at a time when the contractions are only about a quarter of the normal size.

Refractory period. In nitrogen by the time the contractions are reduced to a quarter of the normal there is a marked lengthening of the refractory period. On recovery in oxygen it shortens again. The changes are seen in Fig. 1.

In CO_2 there is a transient shortening of the refractory period occurring soon after the first effects of acid are seen in the form of a slightly diminished contraction. The refractory period generally lengthens again when the contractions are becoming quite small. During recovery in oxygen there is generally a quickening of rhythm which may be maintained for a long time.

At fairly high CO_2 concentrations a muscle stimulated at twice the rate it can initially follow usually breaks from the half rhythm to single rhythm for a time. It is clear that the refractory period must be roughly halved for this to happen. With lower CO_2 concentrations there

was sometimes no visible change by this method except perhaps an occasional extra beat, so that another method was used.

This method was to drive the muscle with a rapid series of shocks only just above threshold strength six to ten times as fast as the muscle could follow. Under these conditions quite a small change in refractory period could produce a change in rhythm. The disadvantage of this method is that the muscle usually gives grouped beats with a slight pause after the group and it is necessary to take an average over a considerable number of beats or else the maximum rate of response.

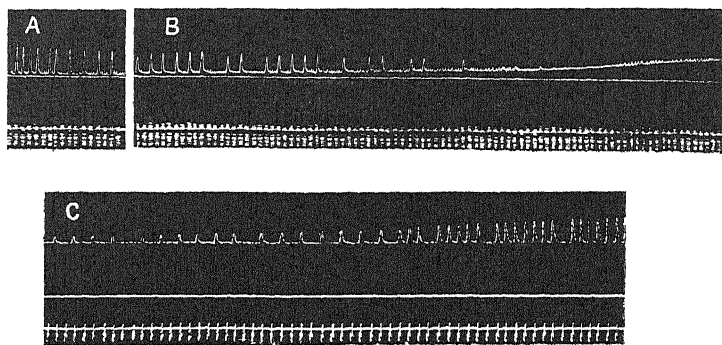


Fig. 1. Refractory period in nitrogen. A and B, ventricle in I.A.A. and N_2 at $0^\circ C$. Lowest line stimuli at 5 sec. intervals, break shocks only. In A, secondary coil at 5 cm. distance. B, a little later coil pushed up. C, another muscle recovering in O_2 . Stimuli at 8.5 sec. intervals. Coil at 5 cm.

In one experiment the muscle was at $0^\circ C$. and was driven by one shock in 2.5 sec., and the average rate counted for about 30 shocks, with results as follows:

Oxygen, pH 8.4 at the beginning, 12 beats per 100 shocks.

Oxygen, pH 8.4 at the end, 15 beats per 100 shocks.

6.4 p.c. CO_2 in O_2 , pH 6.7 at first, 24 beats per 100 shocks, height of contraction 98-74 p.c.

6.4 p.c. CO_2 in O_2 , pH 6.7 later, 9 beats per 100 shocks, height of contraction 67 p.c.

Nitrogen, pH 8.4, 7 beats per 100 shocks, height of contraction 26 p.c.

In another experiment, using 4.8 p.c. CO_2 (pH 6.8), the change was only from an average of 27 beats per hundred shocks to an average of 32. But the maximum rate was 1 beat to 3 shocks in oxygen and 1 beat to 2 shocks in CO_2 .

As regards the primary change of refractory period with change from alkaline to acid conditions, these results merely confirm Adrian's [1920] observations, under different conditions. He perfused a heart with the

ventricle made quiescent by a Stannius ligature with buffered Ringer of varying hydrogen ion concentration. The ventricle was stimulated with pairs of shocks at varying intervals and varying strengths to determine the recovery of excitability. As it was only stimulated occasionally the secondary lengthening of the refractory period was not obtained. He made similar observations on frog's nerve and skeletal muscle and obtained a similar result; in acid conditions there was a shorter refractory period followed by a supernormal phase, in alkaline conditions the refractory period was about twice as long and there was no supernormal phase.

A halving of the refractory period is also about the maximum observed in the present experiments, and it might be objected as against the interpretation put on the result that the *in vitro* change in rate of breakdown of phosphagen as between pH 8.5 and 6.5 is far greater than twice. This objection is not, however, so cogent when it is remembered that there are two antagonistic effects in the muscle, one producing quicker breakdown, the other slowing synthesis and so reducing the concentration.

There is a further piece of evidence that is relevant here. Under any conditions whatever if the ventricle is driven by a rapid series of strong stimuli it gives a series of contractions (three to eight or even more) at a rapid rhythm, then a pause and then another similar series, or else breaks permanently into a slower rhythm. Now on any theory there will be some exhaustion of the phosphagen store at the quickest rhythm, and the pause or slower rhythm that follows must be due to a lengthening of the refractory period. It seems safe to conclude that the rate of breakdown of phosphagen determines the length of the refractory period.

Latent period. A good many experiments were inconclusive on account of progressive changes in viscosity seen as changing rate of contraction and relaxation. But where the viscosity of the muscle working normally in oxygen remained reasonably constant the results were quite clear. Exhaustion in nitrogen produces no change in latent period. In CO₂ there is a lengthening of latent period to be attributed to increased viscosity. The character of the contractions can be seen in Fig. 2.

The results of another experiment are given numerically in Table I. The figures for latent period represent the mean of 2 to 4 consecutive contractions which seldom differed by as much as 0.1 sec.

The fact that the latent period in CO₂ lengthens as soon as any effect is seen and increases regularly with diminishing size of contraction makes

it unlikely that there is a shortening effect at some stage which is masked by increasing viscosity. As far as the results go they lend no support

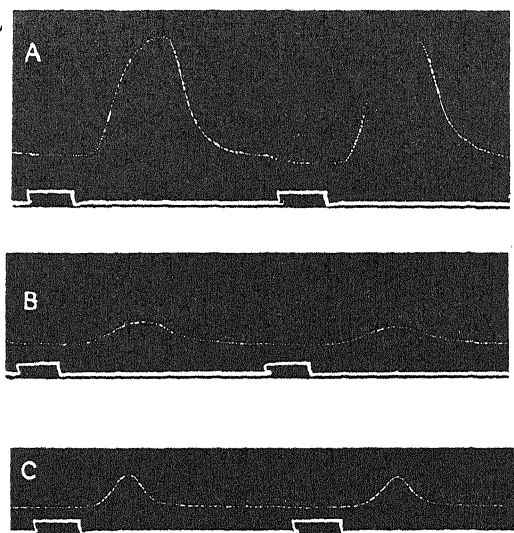


Fig. 2. Latent period. Ventricle in I.A.A. at 0° C. Lower line stimuli at 11 sec. intervals, break shocks only. A, normal beat in O₂, pH 8.4. B, beat failing in 15 p.c. CO₂ in O₂, pH 6.3. C, same muscle in N₂ after recovering in O₂, pH 8.4.

TABLE I.

	Size of contraction as p.c. of initial	Latent periods sec.
Oxygen (pH 8.6)	100	1.1-1.2
CO ₂ 8 p.c. (pH 6.6)	92	1.3
"	84	1.4
"	74	1.7
"	40	2.5
Recovery in oxygen	72	1.0
"	76	1.2
Nitrogen	72	1.1
"	58	1.1
"	36	1.2
"	24	1.1
Recovery in oxygen	56	0.9
"	68	0.9
"	72	1.2

to the view that phosphagen breakdown is the causal process because the predicted effects are not found. But it would not be difficult to explain away this negative result, so that the evidence is not very strong.

DISCUSSION.

The implications of the chemical theory may be considered first. If phosphagen breakdown precedes contraction and liberates the energy needed for it, relaxation might at first sight be supposed to be due to resynthesis of phosphagen. But if this were so the I.A.A. muscle in nitrogen would hardly be able to relax and certainly could not finish up in a relaxed state. As phosphagen breakdown *in vitro* liberates base at reactions more acid than pH 8 [Meyerhof and Lohmann, 1928 b] this might be supposed to be an important factor. Frog's ventricle, however, and skeletal muscle, work well in Ringer between pH 8 and 9 when liberation of base is negligible and give a diminished contraction on the acid side of pH 7, and generally nothing at pH 6 just where the liberation of base ought to be greatest. This supposition is therefore most improbable. In fact, it is not likely that changes of acid-base equilibrium in the tissue are of primary importance. There is no obvious property of phosphagen or its breakdown products to connect them with the production of contraction.

As the time interval between excitation and contraction is almost nil [Roos, 1932] any intervening chemical reaction must be extremely rapid. A velocity comparable to that of ionic reactions might be expected, whereas the breakdown of phosphagen *in vitro* or in minced muscle though rapid compared with hydrolytic reactions of organic compounds is not at all of this order of magnitude.

On this theory the speed of breakdown of phosphagen in different muscles must be fairly closely related to speed of contraction. Considering only vertebrate muscles which have the same phosphagen, frog's skeletal muscle is about 1000 times as quick as tortoise's stomach muscle, but is very much slower than the wing muscles of a humming bird. The variations in concentration of phosphagen are hardly of this order, so that the rate of breakdown must depend upon other factors too, say the concentration of some catalyst. Though a catalyst may keep the speed of reaction constant while there is enough phosphagen to saturate it, at some stage, as the store becomes exhausted, the speed ought to diminish. If we are to explain on this theory how it is that the contractions of the I.A.A. muscle in nitrogen get smaller but not appreciably slower as the store diminishes, further assumptions of a purely gratuitous nature will have to be made.

On the physical theory the correlation of rate of recovery of excitability with the amount of phosphagen does not present such great diffi-

culties. The rate of recovery of excitability diminishes as the store of phosphagen diminishes. As regards the comparison of different muscles, the refractory period of frog's heart is of the order of 100 times as long as that of frog's skeletal muscle. The difference of concentration of phosphagen is not so great. The working level in skeletal muscle is from 65 to 25 mg. per 100 g., that in heart muscle 5 to 1 mg. per 100 g. [Clark, Eggleton and Eggleton, 1932]. The discrepancy does not offer any great difficulty, as the rate of breakdown is not likely to be simply proportional to phosphagen concentration, but more nearly proportional to the product of its concentration and that of some constituent of the catalytic system.

The usual view of the excitation process and its transmission in nerve (and presumably in muscle) is that it consists in the depolarization of a membrane which at rest is kept polarized [see Hill, 1932]. In this process energy is liberated and the subsequent repolarization process involves the performance of work by the cell at the expense of exothermic chemical reactions. The breakdown of phosphagen is supposed to be the first of the exothermic reactions in muscle and probably in nerve [Gerard, 1932] and is likely to provide the immediate source of energy for repolarization. It may be objected that there is nothing known about phosphagen or its products to fit it for this rôle, but on the other hand there is nothing known about any other chemical reaction that could explain any better its ability to play this part. The difficulty is only one special case of the general problem of how cells produce and maintain concentration differences across the cell boundary by means of chemical reactions within. Any reaction producing diffusible ionized products from a unionized or indiffusible precursor is a not impossible means.

In a nerve fibre the chemical reactions are all on a small scale and the potential energy of the polarized membrane is probably also small (*i.e.* small in quantity, not necessarily of small intensity). In muscle the chemical changes are on a large scale and the potential energy of the polarized excitable membrane should be large also. Further, on the present view, some of the energy must be available for the mechanical process as well as simply for excitation and conduction. Unfortunately it is impossible to suppose that the same structure subserves the two functions because if it did there would be a constant relation between the refractory period and the two phases of the mechanical response. In heart there would be no difficulty, for the absolute and relative refractory periods coincide fairly closely with the times of contraction and relaxation; but in skeletal muscle the absolute refractory period is

extremely short, less than 0.01 sec., and the relative refractory period is finished at about the summit of contraction [Adrian, 1921; Hartree and Hill, 1921]. It is necessary therefore to postulate separate structures, one for excitation and one for contraction. While it would be premature to postulate the existence of any particular structural mechanism, among the various alternative possibilities, it seems legitimate to suggest that in effect an electric charge carried by the excitation membrane may be transferred to the contractile structure. Thus there might be (1) a longi-

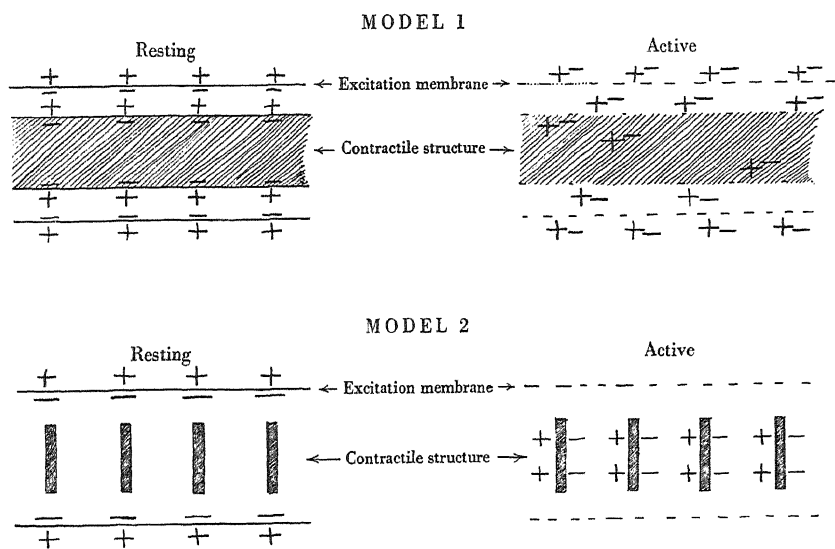


Fig. 3.

tudinally arranged structure expanded in the resting state by carrying an electric charge, and this would be the equilibrium state as long as the excitation membrane remained polarized. On depolarization the charge would be neutralized and the structure acquire a shorter natural length. Or else (2) the contractile structure might consist of transverse plates uncharged at rest and charged up after excitation.

The two models are shown diagrammatically in Fig. 3.

SUMMARY.

1. Assuming that the first known chemical change that occurs in muscle after stimulation is the breakdown of phosphagen, this reaction might be the causal process producing contraction (chemical theory);

or else contraction may be due to liberation of electrical or mechanical potential energy, not to phosphagen breakdown; this reaction then would be the first of the recovery processes by which the potential energy is restored (physical theory).

2. On the physical theory changing the rate of breakdown of phosphagen should alter the duration of the refractory period in the same sense. On the chemical theory it might be expected to alter the duration of the apparent latent period in the same sense, provided other factors remain constant. The rate of breakdown of phosphagen in the iodoacetate muscle can be increased by making the tissue more acid with CO_2 and diminished by exhausting the store in nitrogen.

3. The results of experiments on the ventricle of the frog's heart are in favour of the physical theory and lend no support to the chemical theory.

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THE POLYMORPHONUCLEAR-LYMPHOCYTE RATIO
AT AN ALTITUDE OF 5750 FEET.

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IN view of the importance which may be attached to the differential leucocyte count and the inferences which are sometimes drawn from even slight variations in the polynuclear-lymphocyte ratio, the present state of knowledge regarding the relative proportions of the different kinds of leucocytes which are found in normal blood seems to be somewhat inadequate.

It is not the purpose of this paper to deal with the variations in total white cell count which occur in normal healthy subjects, but it may be mentioned that, from the experience of the past few years in examining young healthy students in this laboratory, the fluctuations are much greater than have hitherto been thought. The records which are given here refer only to the differential count, made over a period of 4 years, upon 171 medical students, of ages varying between 18 and 24 years.

Averages for the figures given by the following text-books: Bainbridge and Menzies, Buckmaster, Burnham, Flack and Hill, Halliburton and McDowall, Howell, Hutchison and Hunter, Schafer, Schliep, Starling, Stitt, Samson Wright, work out as follows: Polymorphs, 68.2 p.c., lymphocytes, 25.8 p.c. It is probable, if not certain, that these figures relate to observations made at sea-level; the references in the literature to counts made at altitudes substantially above sea-level are very scanty. It is, however, a well-established fact that ultra-violet radiation exerts a profound effect upon the differential count. In experimental irradiation with strong sources of ultra-violet rays, notable effects have been produced. For example, Clark [1921] states that direct irradiation of rabbits' ears for 1 hour by an iron arc shows that the far ultra-violet produces a marked lymphocytosis. Hardy [1927] also found that ultra-violet rays shorter than 300μ caused

lymphocytosis in rabbits. Clark [1922], in reviewing the physiological effects of light, states that all published results agree that ultra-violet rays stimulate a lymphocytosis in both men and animals.

Johannesburg, which is situated on the Witwatersrand, Transvaal (Lat. 26°11 S., Long. 28°03 E.), at an altitude of 5750 ft. above sea-level, receives 73 p.c. of the total possible hours of sunshine, as compared with Switzerland, 40 p.c., and London, 29 p.c. Moreover, it is well established that sunlight falling on places at a high altitude is richer in ultra-violet rays than that nearer sea-level, and, further, the nearer the equator a district is situated the greater tendency is there for more light of the shorter wave-lengths to fall upon it. Osborn [1929], in a survey of the ultra-violet content of South African sunlight by the acetone-methylene blue method, found an average intensity in Johannesburg of eight times that of London and nearly three times that of Davos. Thus, it might be expected that a relative lymphocytosis would show itself in the blood of residents in such a district as Johannesburg, and this has been found to be the case. The figures are given in Table I.

TABLE I.

Cells	Range	Mean	Standard deviation	Standard error of mean
Polymorphs	38.0-73.0	54.20	6.670	0.510
Mono and trans.	1.7- 7.5	4.24	1.272	0.097
Lymphocytes	21.0-55.3	39.72	6.190	0.473
Eosinophils	0.2- 7.0	1.88	1.231	0.094
Basophils	0.0- 2.6	0.69	0.548	0.042

As regards technique, the following method gave satisfactory results:

The finger was punctured just above the nail, little or no pressure being used, and the surface of a grease-free slide brought in contact with the exuding blood. The end of another slide of slightly narrower width was then applied at an angle of about 30 degrees to the drop and, when the blood had spread out along the edge, was pushed smoothly and evenly along the surface of the first slide. The film thus formed was allowed to dry thoroughly.

Leishman's stain was used. It was found sufficient to count 300 cells in each slide. There is a distinct liability to error if less than 300 are counted, but little or no increase in accuracy was noted when counts of 400, 500, 600 or 700 were made.

The results given in Table I are shown graphically in Fig. 1, so far as they refer to polymorphs and lymphocytes, which are the only significant variables.

A comparison between these figures and the averages from the twelve authors mentioned is given graphically in Fig. 2.

Since the above work was done, I have had the opportunity of examining a thesis by Sachs [1932] on the autonomic nervous system in

dementia præcox, in the course of which he gives the results of differential counts upon 81 African natives confined at the Pretoria Mental Hospital.

Pretoria (Lat. 25.45 S., Long. 28.11 E.) is situated in the Transvaal, at an altitude of 4392 ft. above sea-level. Sachs' figures for polymorphs

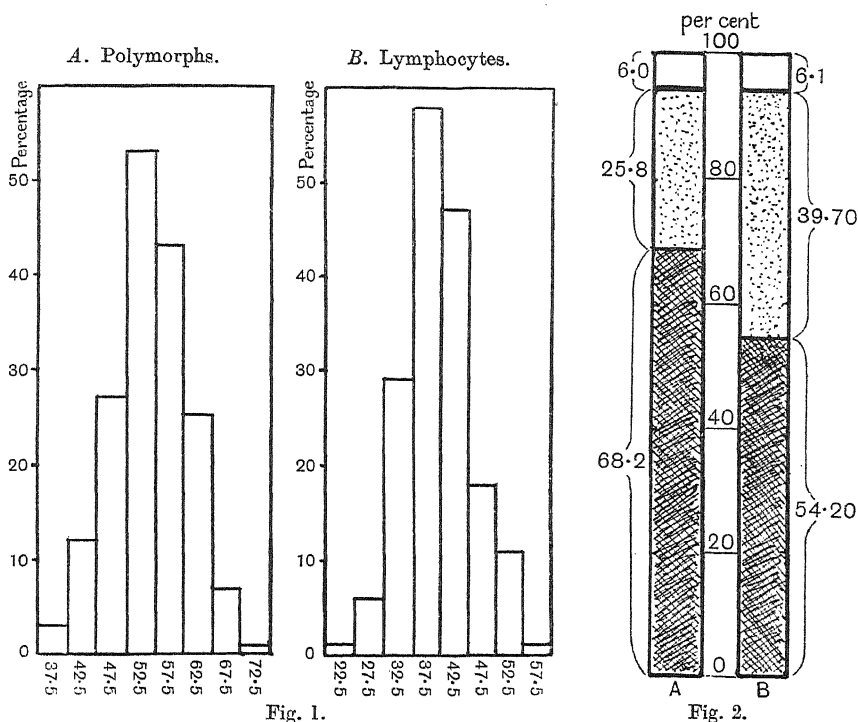


Fig. 1. Ordinates: percentage of total subjects examined. Abscissæ: percentages of polymorphs (A) and lymphocytes (B). A, Mean 54.20. Standard deviation, 6.67. Standard error of mean, 0.51. B, Mean 39.72. Standard deviation, 6.19. Standard error of mean 0.473.

Fig. 2. Darkly shaded areas, Polymorphs. Lightly shaded areas, Lymphocytes. Blank areas, other cells. A, averages of 12 authors (sea-level). B, present investigation (5750 ft.).

and lymphocytes are given in Table II, and it will be seen that there is a close agreement between his figures and my own.

TABLE II.

Cells	Range	Mean	Standard deviation	Standard error of mean
Polymorphs	38.0-72.0	54.70	3.23	0.247
Lymphocytes	21.0-53.0	36.80	7.56	0.578

Since the differential leucocyte count is frequently used as collateral evidence for diagnostic purposes, the significance of the variation from the sea-level figures is likely to be considerable. Hewitt [quoted by Burnham, 1913] states that 75-80 p.c. of polymorphs means that infection is probable, irrespective of the total leucocyte count, and no doubt there will be general agreement that such a percentage is at least suspicious. It only represents, however, an increase of 7-12 p.c. over the normal sea-level figure. A comparable increase over the figure found at Johannesburg gives 61-66 p.c., which is well within the limits of sea-level normality, and is not likely to arouse suspicion, unless the alteration in the ratio at the higher altitude is recognized.

SUMMARY.

1. Analysis of 171 cases of young healthy European adults, living at an altitude of 5750 ft. above sea-level in the Transvaal, reveals a drop in polymorphs of approximately 14 p.c. and a rise in lymphocytes of about the same figure, as compared with the average normals (68.2 and 25.8 p.c. respectively) for sea-level.

2. These findings are confirmed by another worker in the case of a group of 81 natives.

3. In so far as the differential leucocyte count has diagnostic value, the importance of recognizing these variations is obvious.

4. The probable cause is the high degree of ultra-violet radiation which has been established for the locality of the investigation.

I wish to express my thanks to Prof. J. P. Dalton, University of the Witwatersrand, for his assistance with the mathematical portion of this investigation, and also to Dr W. Sachs, for kindly allowing me to make use of his figures.

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THE APPARENT VISCOSITY OF BLOOD FLOWING IN
THE ISOLATED HINDLIMB OF THE DOG, AND
ITS VARIATION WITH CORPUSCULAR
CONCENTRATION.

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"POISEUILLE'S Law was discovered in the course of an investigation preliminary to an understanding of the circulation of the blood in the body" [Barr, 1931]. Poiseuille [1843] confined his well-known observations to the passage of pure liquids through rigid tubes, and this simplification of his problem led to the concept of "the viscosity" as a constant property of a liquid at a given temperature. The return to a direct study of blood was delayed till 1906 when Denning and Watson [1906] found that the apparent viscosity of blood depended on the diameter of the tube in which it was measured, and Hess [1907] found that its apparent viscosity depended on the velocity at which the blood flowed. These disturbing conclusions, based on the passage of blood through rigid tubes, and encompassing only a small range of the variables concerned, rendered the value which the apparent viscosity of blood might attain in the vascular bed quite unpredictable. The present enquiry is directed to the measurement of this value.

The variation of the viscosity of a particular sample of blood has now been shown to be much greater than was at first suspected. Normal blood which according to most text-books of physiology has a viscosity of about 5, may in fact exhibit an apparent viscosity of anything between about 2 and 100 times that of water, depending on the design of the apparatus in which it is measured [Bayliss, 1933]. The usual meaning of the term "viscosity" is inapplicable to such a heterogeneous liquid even as an approximation. We shall follow Barr in adopting "apparent viscosity" to describe the empirical ratio of the volumes of water and blood which would flow in a given time under the same specified conditions, without prejudice to the actual physical explanation

of the variation in internal friction. The term is a variable dependent on (1) the velocity of flow, and (2) the diameter of the tube, in addition to the factors that affect the viscosity of pure liquids; the significance of these two variables in the choice of our experimental conditions may be briefly indicated.

First, decrease of velocity of flow increases the apparent viscosity of blood, as shown by Hess [1907, 1912] and his co-workers over a small range of velocity, and by Bayliss [1933] over a wider range. The change of viscosity with velocity is rapid at low velocities, but becomes less and less as the velocity increases. The apparent viscosity, therefore, approaches a constant minimal value over a considerable range of velocity extending from the low velocities already mentioned to the high velocities at which turbulent motion sets in. The magnitude of the effect is illustrated by an experiment [Bayliss, 1933] in which a sample of blood exhibiting a viscosity of 4.5 at suitable velocities, reached a viscosity of about 100 at very low velocities. In consequence of this property of blood, the Ostwald type of viscometer (low velocity) yields indeterminately high values of viscosity, depending on the exact constants of the particular instrument; in the experiments described below, relative viscosity measurements were made in a horizontal capillary tube 30.3 cm. long and 0.93 mm. diameter, through which blood was driven at pressures varying from 30 to 120 mm. Hg, thus ensuring the relatively high velocities recommended by Hess, but avoiding turbulence.

The value of the viscosity given by such a viscometer is substantially constant over a wide range of velocities, and does not depend on the exact dimensions of the instrument since its bore considerably exceeds the critical value mentioned in the next paragraph. If any practical meaning is to be attached to "the viscosity" of blood, it should as Hess suggests be the value given by such an instrument; but it must be recognized that this value is widely different from that with which one is concerned in the passage of blood through arterioles as will be shown below. Experiments on the influence of the rate of flow on the viscosity have been performed only in relatively large glass tubes and do not therefore enable one to predict the significance of this effect in arterioles under physiological conditions; it is one of the aims of the present investigation to examine this point.

Secondly, three sets of views have been put forward concerning the influence of the diameter of the tube on the apparent viscosity of blood: (1) that the viscosity increases in smaller tubes [Denning and Watson, 1906; and Klisiewicz, 1930], (2) that it is independent of the size of the

tube, [Hess, 1912, and Trevan, 1918], and (3) that it decreases the smaller the tube if the diameter is less than a critical value of about 0.3 mm., but is independent of the bore of the tube if the diameter exceeds this value [Fåhræus and Lindquist, 1931, and Bayliss, 1933].

The latter view, which for reasons given by Fåhræus and Lindquist [1931] seems the most likely, can, however, not be applied to the passage of blood through small blood vessels with any confidence, for these authors find that, whereas the apparent viscosity of a sample of blood in a glass tube of diameter 0.5 mm. was 4.8, and in one of diameter 0.05 mm. was only 3.2, when the diameter was 0.03 mm. or less it was impossible to drive blood through it, indicating an indefinitely high viscosity. A similar block might therefore be expected in the arterioles and capillaries, and such a suspicion would be supported by microscopic observation of asymmetrical distribution of the corpuscles and plasma at the points of bifurcation of the vessels, such as that described as "plasma skimming."

The apparent viscosity of the blood flowing in the arterioles depends therefore on the velocity of flow and the dimensions of the vessels, and possibly on other factors; it is evident that at the moment even the order of its value can hardly be predicted from the results of experiments on blood flow through glass tubes. The present series of experiments is intended to circumvent this difficulty by comparing the rates of flow of blood and Ringer's solution or of blood and plasma in a perfused organ itself; since the viscosity of plasma is substantially independent of the rate of flow and size of tube within the range with which we are concerned, and can moreover be simultaneously measured in the glass viscometer described above, such a comparison is capable of yielding directly the apparent viscosity of the blood under various particular conditions in what might be regarded as a biological viscometer.

Hæmodynamic problems are often concerned with abnormal corpuscular concentrations; for example, general changes in concentration as in anæmia and polycythæmia, or local change such as inflammation or that due to withdrawal of glomerular fluid in the kidney [Winton, 1932] which formed the starting-point of the present enquiry. Our experiments were therefore extended to include the investigation of the influence of corpuscular concentration on the apparent viscosity of blood and the comparison of this effect when the blood flowed in blood vessels and in a glass tube.

THE CHOICE AND PROPERTIES OF A BIOLOGICAL VISCOMETER.

The chief difficulty in determining the apparent viscosity of blood in a living organ is that changes of pressure and composition of the perfusate may change the bore of the perfused blood vessels [cf. Du Bois-Reymond, Brodie and Müller, 1907]. The large arteries are known, from observations on isolated arterial strips, to distend somewhat with increasing pressure, but, since they contribute only a small fraction to the total pressure fall, the changes in them are unlikely to affect the pressure-flow relation sufficiently to be detected. The arterioles are also generally supposed to distend with increasing arterial pressure, a belief based on the pressure-flow relations in isolated organs; Fleisch [1919], for example, has observed a decreasing resistance of the kidney to Ringer perfusion at higher pressures.

No conclusions as to the response of arterioles to pressure can, however, be fairly based on such observations, for the main resistance to Ringer flow in the kidney is not vascular in the ordinary sense but due to compression of the blood vessels by fluid accumulated outside them. When, for example, an isolated kidney perfused with blood from a heart-lung circulation is suddenly submitted to perfusion with oxygenated Ringer at the same temperature and pressure, the flow shows a momentary increase due to the lower viscosity of the Ringer, but, as shown in Fig. 1, this is almost immediately masked by a reduction of flow to a value much below that even of the original blood flow. At the same time the kidney swells and becomes exceedingly turgid; if now the organ be removed from the circulation and cut open, the section exudes water copiously. Vaso-constriction is in general accompanied by shrinkage of the kidney, and is not, therefore, responsible for the sudden restriction of the Ringer flow.

The turgescence of the Ringer-perfused kidney involves a considerable back pressure, and the flow should be proportional to the difference between this and the arterial pressure. Hence, if the arterial pressure only be taken into account, the resistance to Ringer flow appears to decrease with increased pressure, without necessarily deviating from Poiseuille's law or implying change of bore of the vessels. If the kidney were chosen as a viscometer, it would indicate that the viscosity of blood is much less than that of water as shown in Fig. 1. Even if this difficulty could be overcome, the fact that the resistance to blood flow increases with increased blood-pressure in the kidney in which glomerular fluid is formed, whereas it decreases with increased pressure in other

organs [Winton, 1932] would suffice to render the kidney unsuitable as a typical biological viscometer.

We turned our attention, therefore, to the isolated hindlimb of the dog, which can be conveniently perfused through the femoral artery,

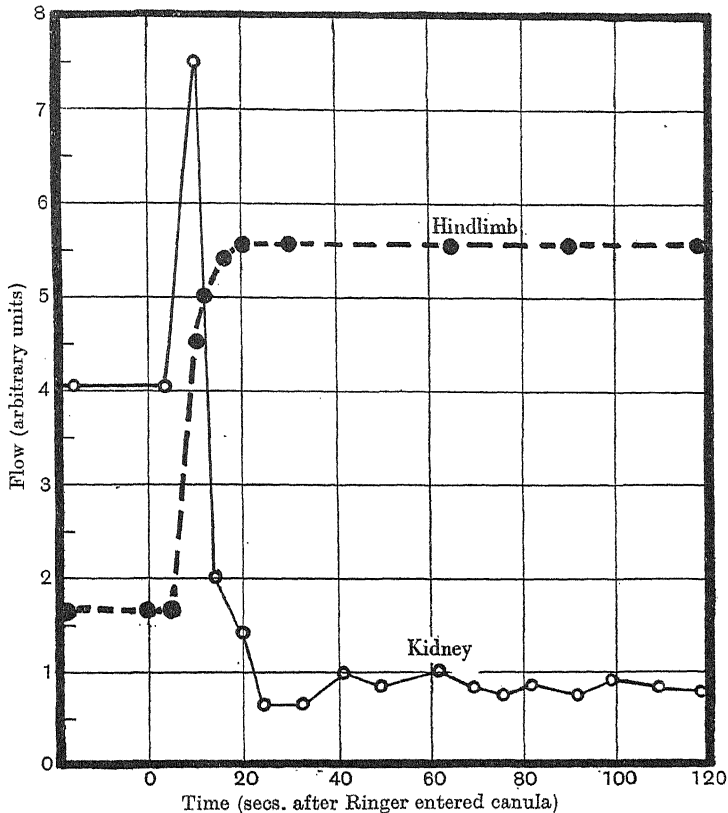


Fig. 1. The variation in perfusion rate at constant pressure during and after the change from blood to Ringer perfusion, in the hindlimb (dots and broken line), and in the kidney (circles and continuous line). The increase of flow is maintained in the limb, but almost immediately abolished in the kidney by the rapid development of a high intrarenal pressure.

liquid from the cut veins being allowed to spill freely into a funnel. If the perfusion fluid in such a preparation be changed suddenly from blood from a heart-lung circuit to oxygenated Ringer's solution at the same temperature and pressure, the flow increases immediately and remains steady, as shown in Fig. 1, often for as long as half an hour or more.

Return to the blood circulation, if not too long delayed, will restore the original blood flow (Fig. 3).

If a hindlimb be perfused with blood or oxygenated Ringer's solution at different pressures, and the values of the flow are measured when a pressure is approached from above and from below, the former value

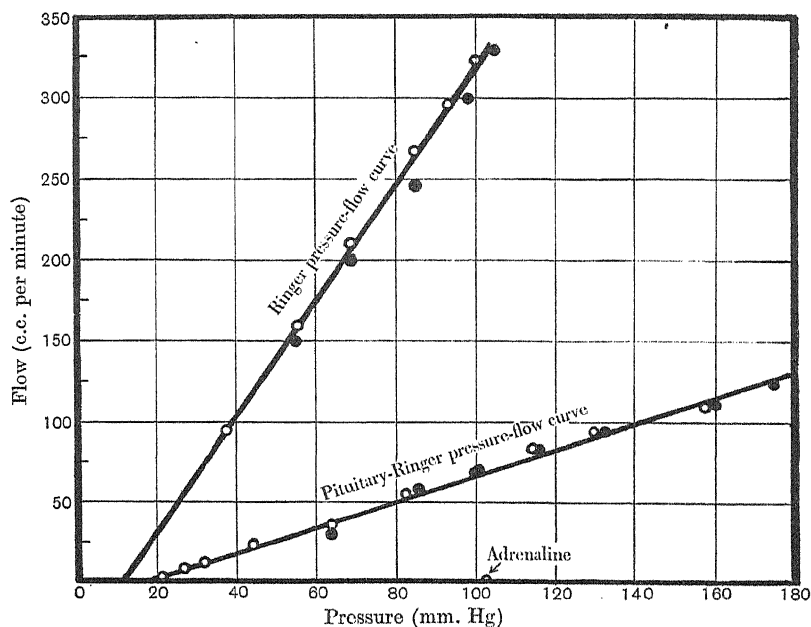


Fig. 2. Ringer pressure-flow curves in a hindlimb, showing (1) that the pressure-flow relation is approximately linear, (2) that this is true even when vaso-constriction is induced by pituitary, (3) that the flow is almost abolished by adrenaline (1 in 10^6), and (4) that the values of the flow obtained when a pressure is approached from below (dots) are usually lower than those obtained by approaching it from above (circles). The larger intercept in the pituitary curve is probably due to the passage of time and associated development of oedema between the measurements recorded in the two curves.

exceeds the latter value by an amount which is less the longer the time allowed for the limb to accommodate itself to the particular pressure (Fig. 2). As, in general, the properties of an isolated limb can be regarded as constant only for about $\frac{1}{2}$ hour during Ringer perfusion, or for 4–5 hours for blood perfusion, and many measurements have to be made in the course of one experiment, it is not feasible to wait for an equilibrium value of flow to be attained at each pressure. Consequently we adopted the procedure of assuming the true flow to be the mean of two values

obtained by approaching the pressure from above and below, and keeping the intervals between the pressure changes approximately the same.

It might be supposed that a hindlimb, isolated as described, would contain anastomoses which would enable some blood to leak out of cut arteries without passing through the vascular bed. It appears that this is not so, for (1) when the pressure in the femoral artery is high, no spurts of blood are projected from the cut surface, (2) the blood emerging from the cut surface appears venous, and (3) adrenaline administered during blood or Ringer perfusion almost obliterates the vascular channels as shown in Fig. 2.

The pressure-flow relation of the limb perfused with Ringer's solution is linear within the limits of experimental error. This remains true if vaso-constriction be induced, as, for example, by post-pituitary extract (Fig. 2). The line does not usually pass through the origin, but cuts the pressure axis between zero and 20 mm. Hg, or occasionally more. This intercept on the pressure axis is probably mainly due to the back pressure exerted by oedema fluid, since (1) it becomes progressively greater the longer the limb is perfused with Ringer's solution, and it becomes much larger if oedema in the limb is allowed to proceed to a degree where it is obvious to the naked eye, (2) oedema in the limb is avoided and can even be removed by blood perfusion [Starling, 1896]; if Ringer perfusion is only a momentary interruption of blood perfusion ("change-over" technique), as in the experiment illustrated in Fig. 3, and a series of such interruptions at different pressures serves to provide a Ringer pressure-flow curve, the intercept is reduced and may vanish, and (3) the pressure of Ringer's solution in the artery must exceed a finite value, usually about one-half that of the intercept, if it is to produce any flow whatever through the limb.

Change in the number of patent vessels and in their bores at different pressures thus plays so small a part in determining the Ringer flow through the limb that the Poiseuille law approximately describes the pressure-flow relation at all but low pressures, if account be taken of the back pressure due to the tissue fluids. This back pressure can only be taken as constant for short periods such as $\frac{1}{2}$ hour, even with 1 p.c. glucose in the Ringer, although oedema visible to the naked eye may not appear for some hours.

The perfusion with defibrinated blood from either a heart-lung or a pump-lung circuit results in a pressure-flow curve consisting again in a surprisingly close approximation to a straight line, when the mean of flows obtained by approaching a pressure from above and below are taken as in Fig. 3. In the blood pressure-flow curve there is also an intercept on the pressure axis, varying in amount between 10 and 30 mm.,

but usually lying somewhere near 20 mm. Hg. In this case, however, the intercept is not primarily due to back pressure, but to a property of blood itself, namely its increasing viscosity at low rates of flow; a similar intercept is found in the pressure-flow curves of blood passing through

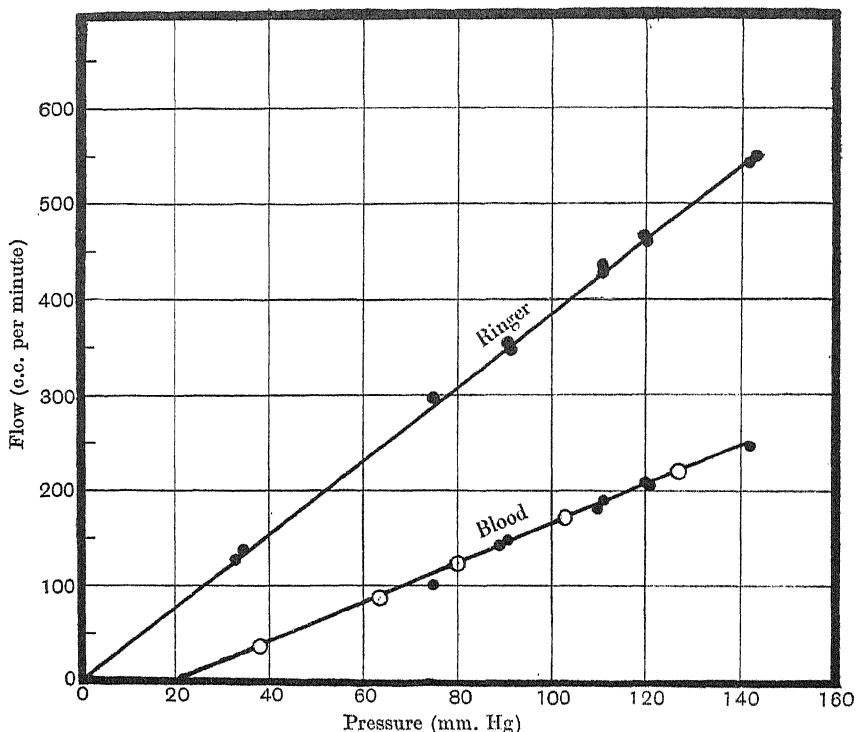


Fig. 3. Pressure-flow curves in a hindlimb, showing (1) that the blood pressure-flow curve is linear and cuts the pressure axis at about 20 mm., (2) that the Ringer pressure-flow curve in the absence of oedema may pass through the origin, and (3) that the ratio of blood and Ringer flow at 90 mm. Hg is about 2.3. This experiment was performed in two parts, (a) the heart-lung perfusion gave the blood pressure-flow curve, the circles being the means of the values obtained when raising and lowering the pressure, and (b) the "change-over" technique: the dot on the blood curve being the blood flow just before changing to Ringer, the two dots on the Ringer curve at the same pressure being successive readings of the flow, the second dot on the blood curve at the same pressure being the blood flow just after the change-over from Ringer.

glass tubes. It is clear then that this intercept is not a fortuitous property of the viscometer but must be counted in assessing the apparent viscosity of blood, which cannot be described only in terms of the slope of the pressure-flow curve. It will be seen below, Fig. 6, that the intercept

increases with increasing corpuscular concentration of the blood. Nevertheless, even with blood perfusion of the limb, the prolonged period after amputation needed for the organ to accommodate itself completely to the artificial circuit is often accompanied by the development of a degree of cedema too small to be observed, and in such cases a few mm. Hg of the intercept are due to the back pressure associated with it. If a fair estimate of the apparent viscosity of blood is to be obtained, therefore, the conditions must be so chosen that the pressure of such a small degree of cedema does not invalidate the result.

In six experiments employing the "change-over" technique illustrated in Fig. 3, the mean intercept on the pressure axis of the Ringer pressure-flow curves was 2 mm. Hg. The intercept of the blood pressure-flow curves exceeded this value by 17 ± 3 mm. Hg. In ten experiments in which the limb was perfused with Ringer throughout the experiment, the intercept was 8 ± 11 mm. Hg. In eleven heart-lung-limb experiments the blood pressure-flow curve showed an intercept of 20 ± 7 mm. Hg.

If the difference in the rate of flow of blood and Ringer through a limb is to be made the basis for an estimate of the viscosity of the blood, the question arises how can one ensure an absence of change in the effective cross-section of the vascular bed when the perfusate is changed? Some limbs are stable (*e.g.* Fig. 1), others show evidence of change of bore of the vessels especially when conditions are not entirely suitable. The discrimination between the two depends on exactly the same kind of observation and argument as is detailed below in connection with Fig. 5.

THE COMPARISON OF BLOOD AND RINGER FLOWS IN THE HINDLIMB.

It would appear then that the apparent viscosity of blood in a hindlimb could be measured by alternate perfusion with blood and Ringer's solution, and a series of such experiments were performed, one of them being represented by Fig. 3. The limb was attached to a heart-lung circulation, and after a period of $1\frac{1}{2}$ hours, during which an equilibrium flow was attained and the preparation became relatively stable, the limb was switched over to oxygenated Ringer's solution at the same temperature for as long as was necessary to wash out all the blood and obtain constant successive readings of the flow. The blood circulation was then at once restored, and measurement of the flow at the same pressure was made. The Ringer's solution consisted of Burn and Dale's [1922] solution modified by the addition of 0.05 p.c. NaHCO_3 and 0.05 p.c. glucose and was aerated by a stream of bubbles of oxygen containing 5 p.c. CO_2 ,

the same gas mixture being employed to ventilate the heart-lung preparation. In some experiments the Ringer was supplied under pulsatile pressure from a pump, in others the pressure was steady; no difference in flow due to presence or absence of pulsation was detected.

Observations of the relative flows of blood and Ringer's solution in six limbs showed that, at 90 mm. Hg. the apparent viscosity of defibrinated blood was 2.2 ± 0.2 times that of Ringer's solution. This was only one-half of the apparent viscosity of the same blood measured in our glass viscometer, which, for reasons already given, yields values of viscosity as low as any viscometer in common use.

Although this technique is feasible, we regarded it as unsuitable for the more extensive series of observations involved in varying the corpuscular concentration of the blood, because (1) the duration of the stable period before the development of significant amount of back pressure is short, (2) better conditions can be devised for ensuring the absence of vaso-constriction or dilatation during change of perfusion medium, and (3) immense quantities of blood are lost in washing through the limb after each Ringer perfusion if dilution of the blood in the heart-lung circuit is to be prevented.

THE TRIPLE PUMP-LUNG-LIMB PERFUSION.

The difficulties inherent in Ringer perfusion can be overcome by perfusing with plasma. Since the plasma obtained by centrifuging defibrinated blood induces vaso-constriction, it must be detoxicated by passage through the lungs. This requires one artificial circulation, and the blood to be compared with it requires a similar circulation. And since we were concerned to examine samples of blood of various corpuscular concentrations, and these had to be ready detoxicated at the appropriate stage of the experiment, at least a third artificial circulation was necessary.

Simultaneous preparation of three heart-lung circuits and their maintenance for 5 or 6 hours with bloods of varying composition appeared a laborious and somewhat hazardous procedure, so we replaced the hearts in all three circuits by "finger-stall pumps" (Fig. 4). We did not detect any difference in the behaviour of the isolated limb when perfused with blood from a heart-lung preparation or from a pump-lung circuit.

The success of the preparation for our purpose depended on the degree of confidence with which we could suppose that the vascular bed of the limb remained unchanged throughout an experiment involving switching to and fro between the different circulations and changes of

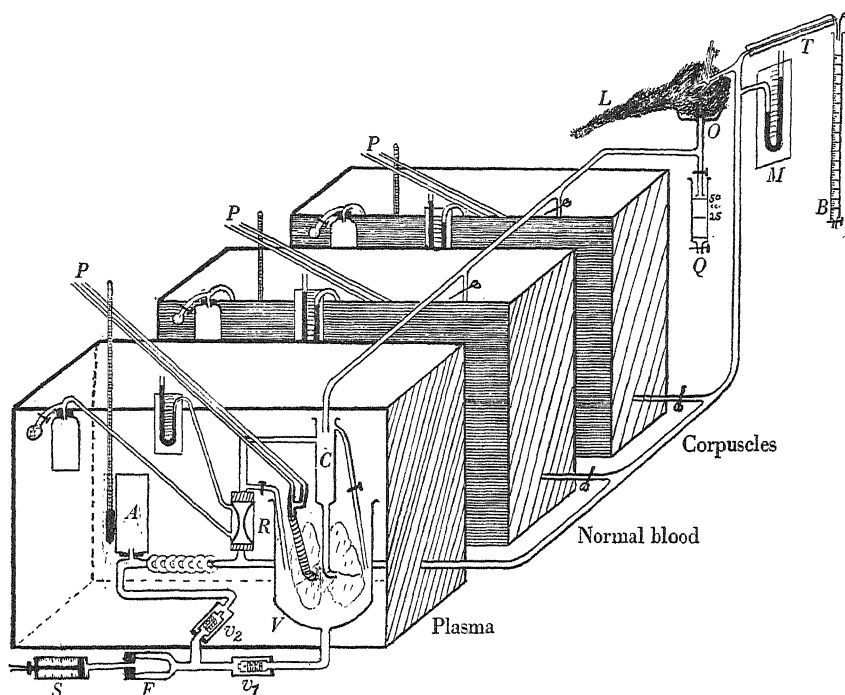


Fig. 4. Triple pump-lung perfusion apparatus. A 10 c.c. syringe (*S*) driven electrically pumps air in and out of finger-stall (*F*). Blood is drawn from venous reservoir (*V*) through valve (v_1), pumped through valve (v_2), and smoothed by air bottle (*A*) and an arterial resistance (*R*) of the type used in heart-lung preparations. Blood either passes out of the tank to the limb (*L*), or escapes through resistance (*R*) to the cannula (*C*) in the pulmonary artery. The cannula (*C*) is wide-bore so as to act as air-vent, and contains an overflow pipe so as to limit the pressure in the pulmonary artery to a maximum of 20 cm. blood. The lungs float in blood in the funnel of a No. 3 Cona Coffee Machine (*V*), and are inflated with 5 p.c. CO_2 in oxygen from tubes (*P*) leading to a Starling "Ideal" respiration pump.

Three such pump-lung circuits in water baths at 40°C . are so arranged that any one of them may supply blood to the femoral artery of the limb (*L*) and to the viscometer (*T*) which are arranged in parallel. Blood from the limb spills into the funnel (*O*) and returns by gravity to the appropriate pulmonary cannula; the blood flow is measured by timing the collection of 50 c.c. in the vessel (*Q*). The viscometer (*T*) is a horizontal capillary tube, 30.3 cm. long and 0.93 mm. diameter, enclosed in a cylindrical glass water bath heated electrically to the temperature of the blood in the femoral cannula; the blood flow through it is timed in the burette (*B*) at the same time as the blood flow through the limb is measured at (*Q*), the arterial pressure for both systems being recorded on the manometer (*M*). The limb, etc., was symmetrically arranged over the central circulation, though displaced to one side in the diagram.

arterial pressure during a period of some 3 hours. Many dogs lost their lives in our attempt to discover the conditions under which vaso-constriction did not result from change of the perfusion fluid, and gradual drift in the rate of flow of the same blood at successive stages of the experiment could be eliminated. Fig. 5 illustrates the changes in flow

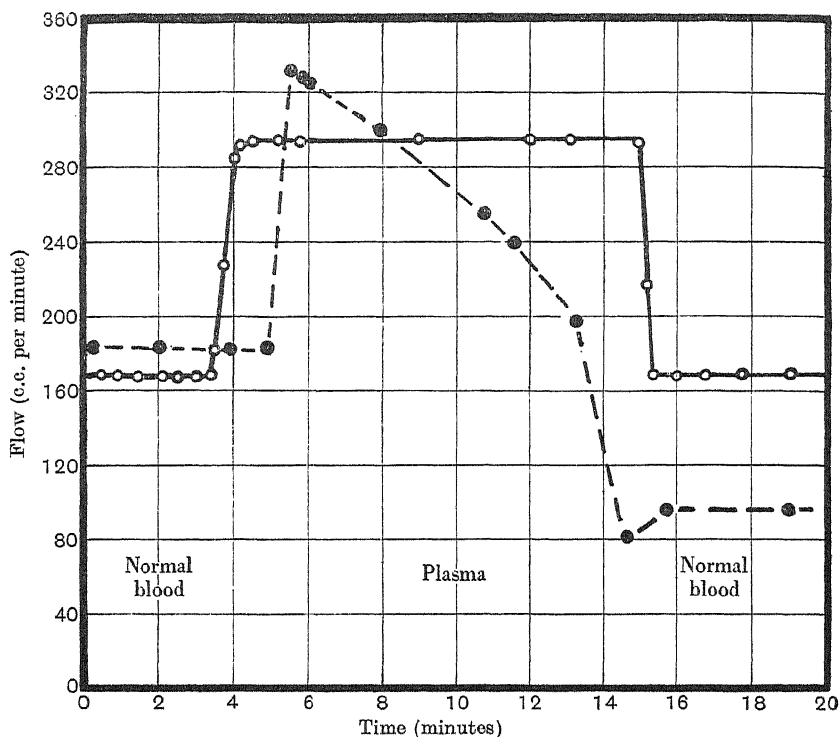


Fig. 5. Curves showing the variations of flow through a hindlimb which result from change of perfusion fluid from blood of normal corpuscular concentration to plasma and back. The continuous line shows the immediate change to the new value of the flow, the maintenance of this value, and the subsequent immediate return to the original value, which is characteristic of a limb in which no change of the vascular bed occurs. The broken line shows the vaso-constrictor response to insufficiently "detoxicated" plasma.

in two limbs when the perfusion fluid was changed from normal blood to plasma and back. In the dotted curve, selected from our preliminary experiments, vaso-constriction is induced by the plasma, and the recovery when normal blood is again used is slow and incomplete. In the unbroken curve, selected from our later experiments, the change in flow is seen immediately to follow the exhibition of plasma, but the flow

remains accurately steady under the new conditions, and returns immediately and exactly to its original value when the normal blood circulation is restored. We wish to lay some stress on the contrast between these two curves as evidence that in our later experiments there was no appreciable change in the vascular bed due to change from one circulation to another. If vaso-constriction is induced by such a stimulus, it occurs slowly enough to be detected in the way indicated by the broken line; the presumption is, therefore, that none occurs in experiments yielding curves of the type shown by the continuous line. The matter might have been tested further by measuring the change of volume of the limb during change of perfusion fluid, but we formed the impression that our plethysmograph exerted enough pressure to impede the outflow of blood from the limb to an extent which increased the tendency to the development of oedema; we therefore rejected the method.

Hardly less important than prevention of immediate vascular responses to changes of perfusion fluid is that of the gradual development of increased resistance during the course of the experiment, due either to progressive vaso-constriction or to the preparation becoming slightly oedematous. Although in the last group of experiments we found the limb accurately stable throughout the 3 hours or so involved in the measurements, we never felt confident enough of achieving this condition to take it for granted. We made a practice, therefore, of calibrating the limb by making a pressure-flow curve with normal blood before employing any abnormal blood, and thereafter repeating the observations with normal blood between every successive variation of perfusion fluid. Only in this way could we know which of our experiments were successful in achieving complete stability of the limb. But the procedure had the further advantage that in those experiments in which a slow change in the vascular bed was found, especially in the later stages of the experiment, it was possible to correct for the change by comparing the flow of the abnormal fluid with that of the mean of the flows of the normal fluid obtained immediately before and after (Table I).

The main factors which seem to promote stability in the isolated limb preparation are (1) reduction to a minimum of the asphyxial period intervening between removal of the limb from the dog and its artificial perfusion; (2) employing for perfusion, blood already detoxicated by circulation through lungs; (3) adding 0.1 p.c. chloral hydrate and 0.4 p.c. glucose to all the perfusion fluids; (4) allowing the limb at least 2 and preferably 3 hours during which to accommodate itself to the conditions of artificial perfusion, before beginning measurements. Lest it be supposed

that long-continued perfusion in the presence of a narcotic kills the limb, it may be observed that a well-marked vaso-constrictor response to one in one hundred million adrenaline can be obtained after about 7 hours' perfusion, and there is no reason to suppose that normal vascular responses could not be elicited for a longer period. The termination of a successful experiment is due to the fact that deliberate mixing of the abnormal bloods has left one only with normal blood. A few experiments were brought to an end by the onset of oedema in the lungs, and a few by failure of one or other of some 260 glass-rubber connections, the secure assembly of which, after the cleaning by boiling which preceded each experiment, is one of the difficulties of the experiment.

THE TECHNIQUE OF THE DETERMINATION OF BLOOD VISCOSITY IN THE LIMB.

The principle of the blood-viscosity measurement is to compare the rates of flow of a sample of blood and of plasma through the same limb at the same arterial pressure. The perfusion fluids are supplied at the same pressure so that the degree of passive distension of the vascular bed may be the same, and also that if there be any back pressure due to oedema, its absolute magnitude has no effect on the value of the viscosity obtained, which depends only on the ratio of two flows.

The outline of the experimental procedure is as follows:

A. *Making the triple pump-lung-limb preparation*: (1) About 2600 c.c. of defibrinated blood are obtained from the requisite number of dogs and mixed. About 1200 c.c. are reserved as normal blood, the remainder being centrifuged to obtain "plasma" and "corpuscles." (2) The lungs from one dog are cannulated, the coagulable blood in them washed out with defibrinated blood, and the artificial circulation of "normal blood" is set going. (3) After an interval of half an hour or so, a hindlimb is removed from another dog, and the femoral artery attached to the perfusion apparatus; about 150 c.c. of defibrinated blood are employed to wash the coagulable blood out of it, and it is then left for 2-3 hours, being perfused with the defibrinated blood at about 110 mm. Hg. (4) The lungs from the last dog are cannulated, washed through with plasma prepared from the defibrinated blood, and the plasma circulation is set going. (5) Similarly the lungs from another dog are cannulated, washed through with "corpuscles" and then incorporated in the artificial "corpuscle" circulation. All perfusion fluids contain 0.1 p.c. chloral hydrate and 0.4 p.c. glucose.

B. *The blood-flow measurements* are conveniently described in terms of a particular experiment (Fig. 6): (1) Preliminary blood-flow measurements were made at intervals during about half an hour to discover

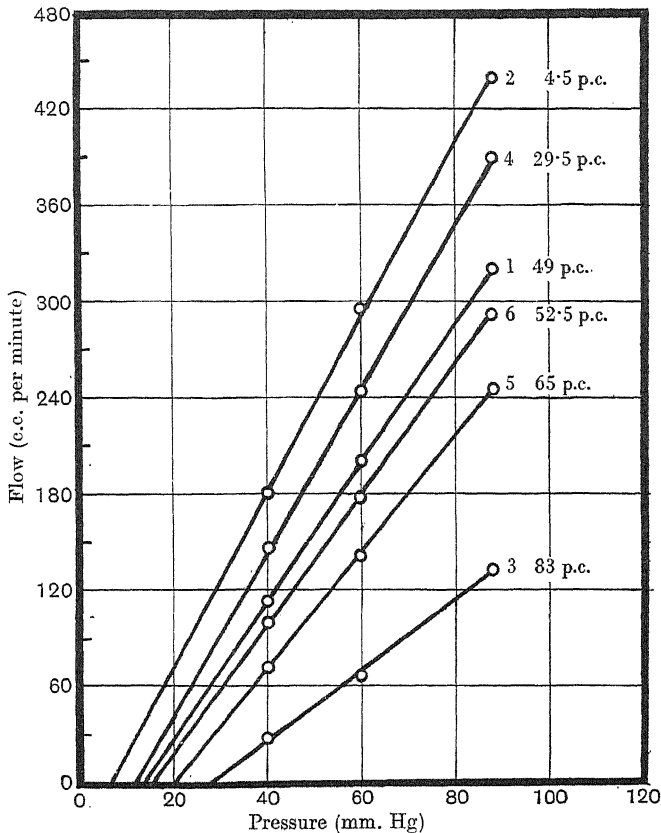


Fig. 6. Pressure-flow curves in a hindlimb with bloods of corpuscular concentration varying from 4.5 to 83 p.c. as shown. They indicate (1) that the curves are linear, (2) that they intersect the pressure axis at values increasing with corpuscular concentration, and (3) how the viscosity determinations were performed (see text). The numbers of the curves indicate the order in which they were obtained, except No. 1 which represents superimposed curves obtained before and after each of the other curves. Each curve represents the mean of values obtained during raising and lowering of the pressure.

whether the limb was in a stable state. (2) A pressure-flow curve for normal blood was obtained (curve 1), each point on the curve representing the mean of the values of the flow obtained by approaching the pressure from above and below, and each such value being the mean of about

three observations. (3) Throughout the experiment, whenever a flow determination was made on the limb by one observer, a simultaneous flow determination was made on the glass viscometer by another observer, thus ensuring that the pressure and composition of the blood should be the same. (4) During the pressure-flow determination for any given sample of perfusion fluid, a few cubic centimetres were taken for subsequent hæmatocrit tests. (5) The circulation was then switched over to the plasma circulation; about 120 c.c. of plasma were passed through the limb to wash out the normal blood and separately collected as "mixed blood" before the outflow from the limb was allowed to return to the lungs in the plasma circulation. Even by so washing out the limb, having already washed out the lungs as described above, it was not possible to prevent some residual corpuscles from contaminating the plasma. The hæmatocrit value of our plasma circulation at this stage varied in different experiments from about 2 to 4 p.c., and the correction for this will be discussed below. (6) A pressure-flow curve for plasma was obtained by the same technique and at the same pressures as for the normal blood. This is shown in Fig. 6 as curve 2. (7) The circulation was switched back to normal blood, a separate collection of "mixed blood" preventing contamination of the normal blood with plasma. This and the previous collection of mixed blood were added to the plasma circulation, to prevent them becoming cold and toxic, and to increase the corpuscular concentration in this circulation to a suitable value for a subsequent test. The pressure-flow curve for normal blood was re-determined, and coincided with curve 1. (8) The limb and viscometer were switched over to the corpuscle circulation, contamination of which was prevented as before. The flow at the different pressures used before were determined and yielded curve 3. The circulation was switched back to normal blood, with the usual precautions. A quantity of fluid was abstracted from the plasma circulation and added to the corpuscle circulation so as to dilute it somewhat for future use. The mixed bloods collected during the change to and from the corpuscle circulation, together with some concentrated blood from the corpuscle circulation, were added to the plasma circulation, so as to concentrate it somewhat further. The pressure-flow curve taken with the normal blood at this stage again coincided with the original curve 1 in Fig. 6. (9) The fluid now in the plasma circulation was tested as before, and yielded the pressure-flow curve No. 4. (10) The normal blood pressure-flow curve was again taken and found to be unchanged. (11) The fluid now in the corpuscle circulation was examined as before, and yielded the pressure-flow curve No. 5. (12) A final re-

determination of the pressure-flow curve for normal blood still coincided with the original curve 1, showing that this had been one of the favourable experiments in which the control had been uncomplicated either by change in the vascular resistance of the limb or by withdrawal of fluid from the blood by the lungs. (13) The remaining fluid in the plasma circulation was added to that in the corpuscle circulation in an attempt to make its corpuscular concentration approach that of the normal blood. If a fortuitous difference in composition of the abnormal bloods had occurred, and was sufficient to induce a vascular response of the limb, this could be detected by finding the blood flow of the normal blood and the mixed abnormal bloods to be different. We found no evidence of such a difference in our successful experiments, though we were usually unable to hit off exactly the same corpuscular concentrations; Fig. 6, curve 6, represents the pressure-flow curve of the mixed abnormal bloods, and if the values be replotted to show the relation between corpuscular concentration and blood flow at each pressure there is no discontinuity between the values of curve 1 and those of curve 6.

The experiment illustrated by Fig. 6 is an example of the most favourable group of our series. Several experiments differed from it only in that our estimate of the probable duration of the stable period of the preparation did not justify us in making flow measurements at more than two pressures, and in some of these when we found we had underestimated the longevity of the preparation we varied the procedure by examining a greater variety of bloods with different corpuscular concentrations (*e.g.* Fig. 7). A few experiments were carried out at one pressure only. In five experiments our re-determinations of the pressure-flow curves with normal blood satisfied us that no appreciable deviation of the properties of the limb had complicated our results, the change of the control flow being less than 2 p.c. between the beginning and end of the experiment. In six experiments a small progressive deviation occurred, and this was corrected for proportionately according to the change of flow of normal blood at the particular pressure (*e.g.* Table I). Many experiments were rejected on account of undue instability of the base-line, the proportion of these becoming rather unaccountably smaller till the last four experiments which required no correction whatever.

Table I gives the data for one of the experiments that required such correction, and is a sufficient description as to how the correction was carried out and the extent to which it modified the results. In deciding whether a value required correction we ignored a deviation of the control flow which was less than 2 p.c.; if it exceeded this value, the abnormal

TABLE I. Observations on hindlimb perfused by triple pump-lung preparation (37° C., 66 mm. Hg). (Figures in brackets are calculated means.)

Time hr. min.	Normal blood		Abnormal blood			Apparent viscosity
	Corpus- cular con- centration (p.c.)	Flow (c.c. per min.)	Corpus- cular con- centration (p.c.)	Flow (c.c. per min.)	Corrected flow (c.c. per min.)	
3 8	47	256				
3 16			2.5	342	342	1.5
3 20	47	253				
3 38			79	110	110	4.7
3 47	47.5	256				
3 56			17	330	330	1.56
4 6	47.0	254				
4 14			58.5	208	208	2.47
4 20	47.0	254				
4 26			27.9	312	312	1.66
4 35	—	250				
4 55		(245)	50	222	230	2.25
5 1	48.0	240				
5 16		(241)	32	297	315	1.64
5 26	48.0	242				
5 35		(243)	48.5	233	243	2.14
5 45		(243)	39.5	263	276	1.86
5 57	47.5	244				

flow was multiplied by a factor equal to the ratio of the mean of the flows of normal blood immediately before and after to the original standard value of the flow of normal blood—all flows being at the same arterial pressure.

All our hæmatocrit determinations were done by centrifuging at least two samples of the same blood in capillary tubes for 1 hour at 3500 revolutions per minute.

EXPERIMENTAL RESULTS.

The measurements described enabled us to construct for each experiment, and at each pressure, a pair of curves showing the relations between the apparent viscosity of the blood and its corpuscular concentration both in the limb and in the glass viscometer; Fig. 7 includes two such pairs, chosen to indicate the extreme range of variation encountered in different experiments. Each curve was constructed in principle by taking the ratio of plasma flow to blood flow and multiplying by 1.5. In practice, as has been stated, pure plasma was not available, the lowest corpuscular concentrations measured being between 2 and 4 p.c.; it was necessary therefore to discover what the flow of pure

plasma would have been by extrapolation of the curves. As will be seen, the curve for the limb is so flat at low corpuscular concentrations

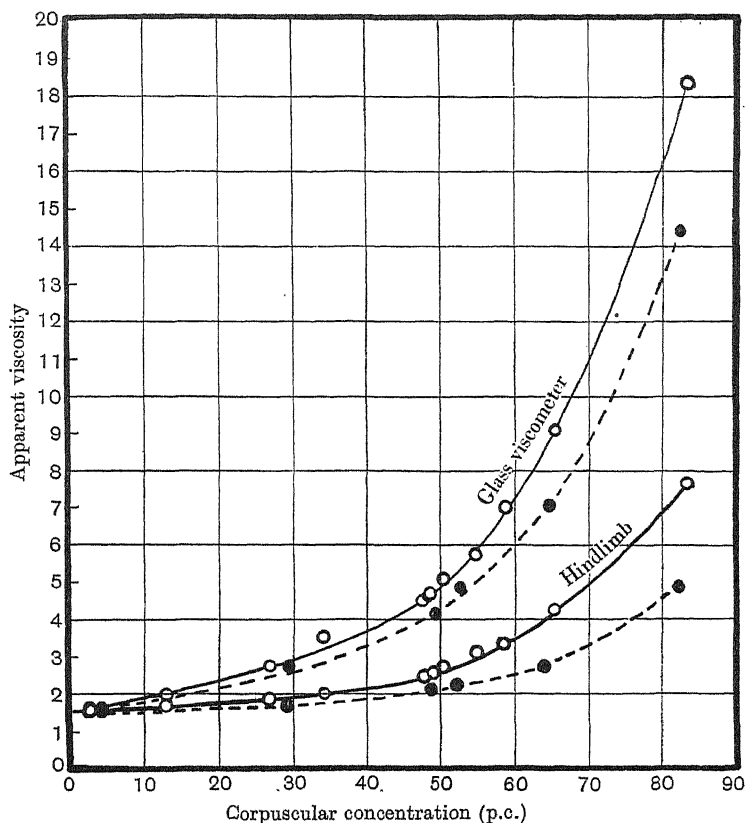


Fig. 7. The relation between corpuscular concentration and the apparent viscosity in the glass viscometer and the hindlimb. Two such pairs of curves, obtained from two different experiments, are shown to indicate the extreme range of variation encountered in our series. The curves are intended to show (1) the approximation of individual experimental values of the viscosity to a smooth curve, (2) that increase of corpuscular concentration increases the range of variation of the viscosity, and (3) that blood of a given corpuscular concentration may deviate considerably from the average viscosity for that concentration. *Note:* Exp. 1 is described by circles and a continuous line, Exp. 2 by dots and a broken line.

that no appreciable error is introduced by taking the flows at zero corpuscular concentration and at, say, 3 p.c. as the same. The glass viscometer curve, however, is steeper in this region. Calibration of the viscometer with water showed that the actual viscosities of the bloods

with the lowest available concentrations of corpuscles were all about 1.6, variations in different experiments being negligible, but the shape of the curve indicated that pure plasma would have had a viscosity of 1.5. In effect, then, the reduction factor for the limb curve was obtained by scaling the flow of our actual "plasma" circulation to 1.5, whereas the factor for the viscometer curve was obtained by scaling the flow of the same fluid down to 1.6.

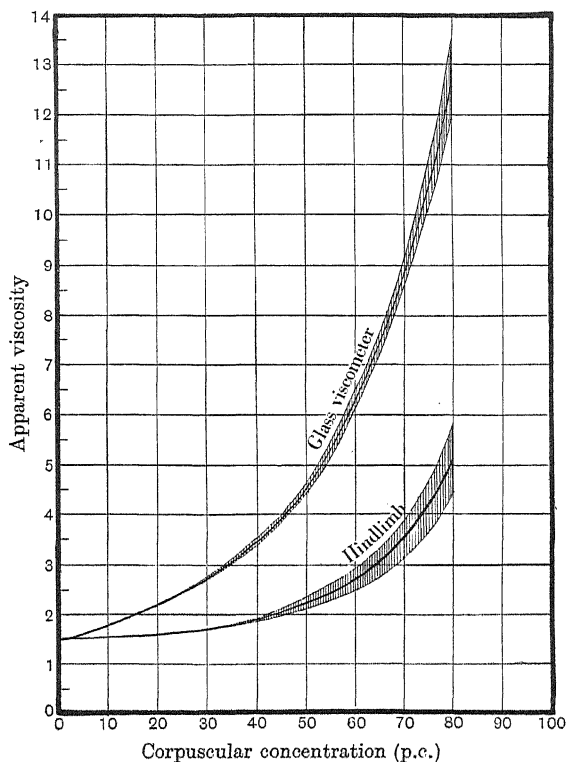


Fig. 8. The mean value and the probable error of the apparent viscosity of blood in the glass viscometer and the hindlimb at different corpuscular concentrations (dog's defibrinated blood at 37° C.).

Fig. 7 illustrates that the experimental values of the apparent viscosity in a given limb fall on a smooth curve, although the corpuscular concentrations were changed in different haphazard orders in different experiments; this would be unlikely to happen unless the bore of the blood vessels remained unaffected.

It will be observed that the curves in Fig. 7 indicate a greater range of variation of viscosity, both in the tube and the limb, as the corpuscular concentration increases. It is not possible to be sure that our precautions against error due to sedimentation in our artificial circulations were completely successful when the rates of flow in the tubes were reduced during the flow of the "corpuscle" circulation, but it seems likely that such differences of viscosity as undoubtedly exist in different samples of blood of the same hæmatocrit value become larger the higher the corpuscular concentration. The circles in Fig. 7 illustrate that when a sample of blood exhibited a relatively high viscosity in the glass tube, it often deviated from the average in the same sense when flowing through the limb. The dots show the same resemblance between the properties of the limb and tube for deviations of the viscosity below the average.

Fig. 8 summarizes the results of eleven pairs of curves, and shows the influence of the corpuscular concentration on the apparent viscosity, described in terms of a weighted mean and probable error. Recording the viscosity in this way will make it possible to compare these observations on the hindlimb with observations on other organs.

The influence of the perfusion pressure on the apparent viscosity of defibrinated blood of normal corpuscular concentration is shown in

TABLE II. The influence of rate of blood flow on the apparent viscosity of normal defibrinated blood (48 p.c.) in the limb and glass viscometer. (Values starred are at pressures not exceeding 50 mm. Hg. Experimental uncertainty is recorded as the standard deviation.)

Exp.	Pressure mm. Hg	Apparent viscosity	
		Limb	Tube
I	80	2.05	4.00
	60	2.15	4.00
	40	2.25*	4.10*
II	120	2.00	4.10
	90	1.95	4.20
	60	1.90	4.25
III	86	2.40	4.30
	50	2.45*	4.40*
IV	68	2.00	4.50
	42	2.50*	4.70*
V	110	2.50	4.90
VI	60	2.35	4.95
	30	2.70*	4.95*
VII	120	2.50	4.40
	90	2.40	4.45
	63	2.45	4.60
Mean	>51	2.2 ± 0.2	4.4 ± 0.3
Mean	<51	2.5 ± 0.2	4.5 ± 0.3

Table II. In the limb whatever effect there may be above 50 mm. Hg is well within the range of our experimental uncertainty which may be indicated by the probable error of 6 p.c. of the mean. The mean viscosity for blood in the limb above 50 mm. Hg is 2.2, the values for pressures lower than this give a mean of 2.5. Scrutiny of the figures in the individual experiments will show that an increase of viscosity at these lower pressures is likely, but within the range of pressure we have explored the change is too small to be of much physiological significance. The mean of the viscosity measurements in the glass tube is 4.45—almost exactly double that of the measurements in the limb. Lower pressures than those given in the table were not employed because they appeared to produce irreversible, or only very slowly reversible, changes in the limb.

It seems evident that the dimensions of the vascular bed in the isolated limb are such that variation of blood-pressure within a physiological range produces no change in the apparent viscosity of blood of a magnitude such that the consequences would be comparable with a small vasomotor change. It is possible, however, that in an innervated limb conditions may arise in which the rate of flow in the significant vascular elements is reduced much further than in our experiments, and if so, a considerable increase of apparent viscosity may be expected. Proof of this must await observations on the effect of vaso-constriction on the apparent viscosity of blood. The only evidence in our series of experiments bearing on this problem is due to the fortuitous circumstance that we found variations of blood flow (from 80 to 400 c.c./min. at 100 mm. Hg) under similar conditions in different limbs, and those variations were in no way correlated with the variations in the apparent viscosity of blood measured in those limbs, even if the weights of the limbs were taken into account. (The dogs used weighed from 8 to 10 kg.)

As stated above, small divergences in the apparent viscosity of normal blood may become magnified when the corpuscular concentration is increased, and Table III may be quoted as another example. This experiment was technically perhaps the most satisfactory of our series, but the influence of blood-pressure on blood viscosity is rather greater than the average of our other experiments. It suggests that the increase of viscosity at low blood-pressures may become a matter of much greater physiological significance if the corpuscular concentration is high than if the blood has a normal concentration. The stasis of the blood in the vessels during inflammation may thus be due to an extremely high value of the viscosity associated with the low velocity and the increase of corpuscular concentration of the blood.

TABLE III. The influence of the rate of flow on the apparent viscosity of blood in a dog's hindlimb.

Corpuscular concentration p.c.	Apparent viscosity at arterial pressures		
	88 mm.	60 mm.	40 mm.
4.5	1.5	1.5	1.5
29.5	1.7	1.8	1.8
49.0	2.1	2.2	2.3
52.5	2.3	2.5	2.7
65.0	2.7	3.1	3.7
83.0	5.0	6.5	9.5

DISCUSSION.

We may conclude from these experiments that the apparent viscosity of blood flowing in the blood vessels of the hindlimb is about one-half that of the same blood flowing in the glass viscometer, although the viscometer is of the high velocity type which yields lower values of the viscosity than the other types which have been used.

The influence of the properties of the viscometer on the apparent viscosity of blood over a wide range of corpuscular concentration is shown in Fig. 9. The three curves represent the relations between the corpuscular concentration and the apparent viscosity of dog's defibrinated blood flowing in (a) an Ostwald type viscometer, (b) our own glass viscometer, and (c) the hindlimb. The first curve is drawn from the data of Trevan [1918]; for values of the corpuscular concentrations (ϕ) exceeding 45 p.c., his observations agree within ± 1 p.c. with Hatschek's equation:

$$\eta = \frac{\eta_0}{1 - \phi^{\frac{2}{3}}}, \quad \dots\dots(1)^1$$

where η is the viscosity of the blood, and η_0 that of the plasma. The application of this equation to our own observations will be considered in connection with Fig. 11. The second and third curves in Fig. 9 summarize the present series of experiments; each point is a weighted mean derived from all the relevant observations we have made.

The simplest explanation of the low values of the apparent viscosity of blood in the limb is based on the well-known microscopic observation, that corpuscles congregate near the axis of a tube, leaving a marginal zone of relatively clear plasma. Most of the relative movement of the concentric zones of liquid occurs in this marginal zone, and hence the greater the proportion of the cross-section of the tube occupied by the relatively corpuscle-free zone, the more closely the apparent viscosity of

¹ In equations (1) and (2) ϕ denotes corpuscular concentration expressed as a fraction. Elsewhere the concentration is expressed as a percentage.

blood approaches that of plasma. The occurrence of an almost solid rod of corpuscles passing down the axis of the tube at a higher velocity than that of the surrounding plasma, implies that the actual average composition of the blood in the tube must be less than that of the composition of the blood in the reservoir supplying it. This change in concentration has been measured by Fåhræus [1929], and contributes towards the lowered viscosity of blood streaming in tubes.

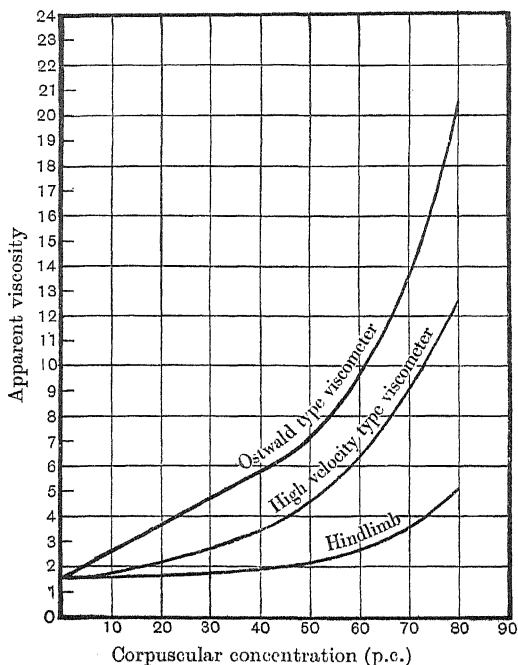


Fig. 9. Curves illustrating the influence of the nature of the viscometer on the apparent viscosity of dog's defibrinated blood at different corpuscular concentrations, 37° C.

If on entering the capillary tube an average corpuscle moves towards the centre at a speed more or less independent of the size of the capillary the proportion of the cross-section of the tube cleared of corpuscles will be greater in smaller tubes than in larger ones, and hence a lower apparent viscosity might be expected in smaller tubes. Moreover, since this centripetal movement is dependent on the flow of the blood—being absent when the blood is stationary—its velocity is presumably higher the greater the rate of flow of the blood; thus at higher rates of blood flow the proportion of a particular tube cleared of corpuscles should be

higher, and the apparent viscosity lower. Such an hypothesis would account for the variation in apparent viscosity observed in glass tubes in consequence of variation of the rate of flow and the diameter of the tube. The viscosity in the limb, though lower than any published result in a glass tube, can be accounted for in the same way, since the arterioles in which the main pressure fall occurs are of smaller bore than the smallest glass tubes in which those results were obtained.

The relative contributions of the uneven distribution of corpuscles, and of the rigidity attributed to certain colloidal solutions by Hess [1912, 1920] and others, to the variation in the apparent viscosity of blood remain to be determined. Both factors would, however, result in a somewhat similar distribution of apparent viscosity in the tube, that near the periphery being lowest, and that near the centre, where the velocity gradient is lowest, being so high that the cylindrical core may be regarded as moving along as a solid rod. Such an arrangement results in blood traversing the tube with a smaller loss of energy than if the viscosity remained evenly distributed; this economy of energy may be related to the force controlling the rate of axial concentration of the corpuscles.

Fåhræus and Lindquist [1931] found that diminution of the bore of a small glass tube reduced the apparent viscosity of blood, but they were unable to reduce it below about 3 owing to difficulties in inducing blood to pass through tubes smaller than 30μ diameter presumably due to agglutination. No such difficulties are encountered in perfusing the hindlimb with blood "detoxicated" by passage through lungs, unless the small variations in the viscosity of bloods of the same corpuscular concentration in different experiments be attributed to differences in aggregation of the corpuscles. Dr L. E. Bayliss informs us that he has succeeded in overcoming the difficulties with glass tubes to the extent of measuring the apparent viscosity of blood in tubes as small as 20μ in diameter; it is a matter of considerable interest that he finds the viscosity in such tubes is about the same as we find in the hindlimb in which the significant vascular elements, the arterioles, are reputed to be of the same order of size. The unexpectedly low value of the viscosity of blood in the hindlimb can, therefore, be accounted for in terms of the relation between viscosity and dimensions of a tube established in experiments on glass tubes. Branching of the vessels, "plasma-skimming," blocking of capillaries by corpuscles, and other anomalies which have been suspected, contribute nothing of physiological significance to resistance of the vascular bed to blood flow.

The possibility that the blood flow in the capillaries is so slow that there is a considerable increase of blood viscosity from this cause is excluded by our experiments, showing that the apparent viscosity of blood in the limb is substantially independent of arterial pressure within the physiological range. This inference depends on the observation that the apparent viscosity of blood is only independent of the velocity when the viscosity assumes its minimal value; if the viscosity in the limb were higher than this value owing to the rate of flow being low, the rate of change of viscosity with velocity would also be high.

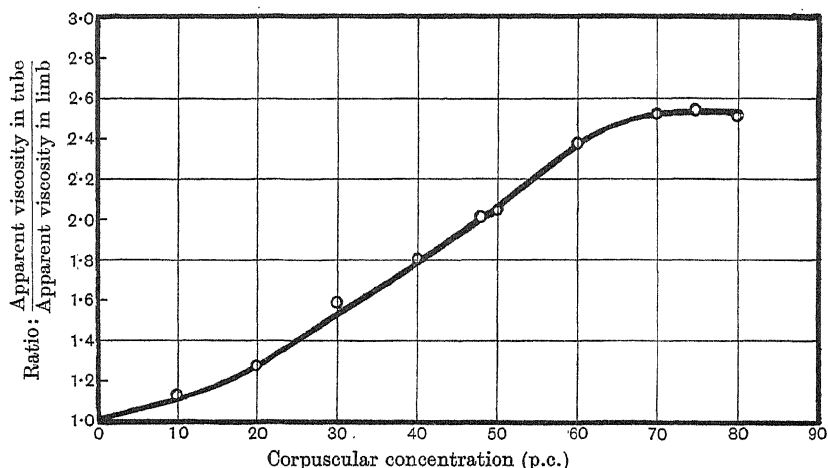


Fig. 10. The ratio of the apparent viscosity of dog's defibrinated blood in the glass viscometer (high velocity type) to that in the hindlimb, at different corpuscular concentrations.

Fig. 10 shows the ratios of the apparent viscosity in the limb to those of the same blood in the viscometer, at different corpuscular concentrations. The ratio for plasma is arbitrarily made unity, for reasons already discussed. Over a range of from 15 to 65 p.c. the ratio increases with corpuscular concentration in an approximately linear fashion. If the viscosity of a sample of blood has been determined in a high velocity type of glass viscometer, this curve enables one to estimate approximately what the apparent viscosity would be if the same blood were flowing in the vascular bed.

In the application of our results to physiological problems one is usually concerned with the change in resistance to blood flow due to change in corpuscular concentration, rather than with the actual values

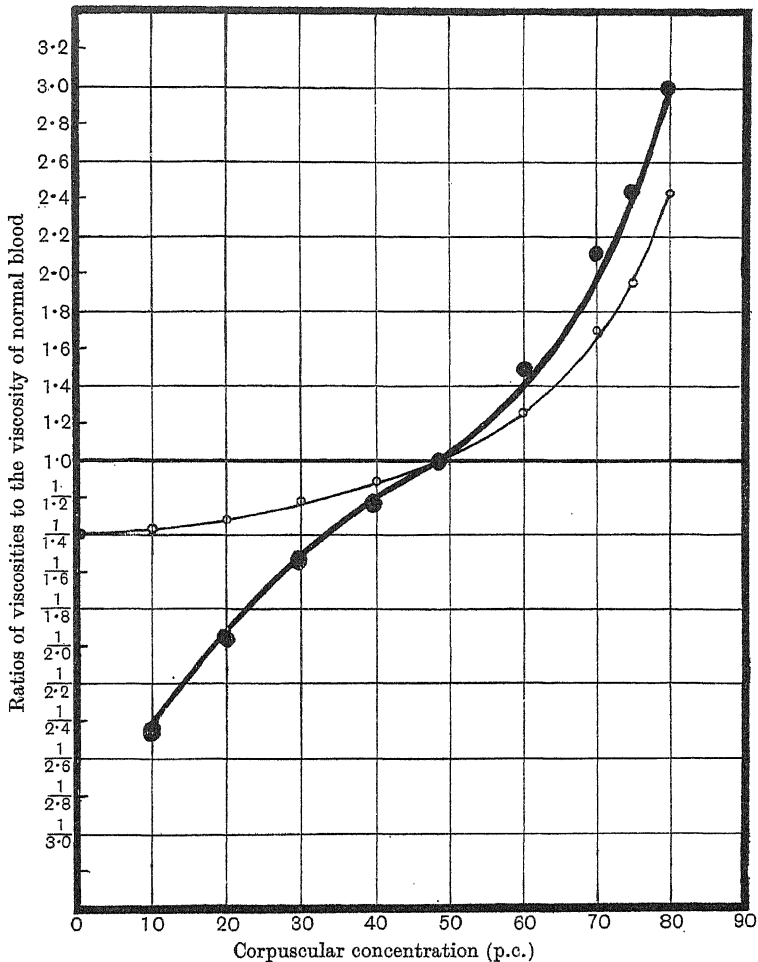


Fig. 11. The apparent viscosity of normal blood (48 p.c. corpuscles) is taken as unity, and the viscosities of bloods of abnormal corpuscular concentration are plotted as the ratios of their actual values to that of normal blood. The thick line denotes the empirical equation (1) in text, agrees with Trevan's observations for corpuscular concentrations exceeding 45 p.c. made with an Ostwald type viscometer, and implies an actual viscosity of normal blood of 7. The dots represent observations made with our high-velocity type viscometer, giving a viscosity of normal blood of 4.4. The circles and thin line represent observations on the hindlimb, giving a viscosity of normal blood of 2.2. *Note:* (1) the approximation of the dots to the thick line, and (2) the divergence of the thick and thin lines.

of the viscosity. It is of interest, therefore, to plot the ratio of the apparent viscosity at any corpuscular concentration to that of normal blood and this is done in Fig. 11. The values (dots) so obtained for our glass viscometer show a curiously exact correspondence to the equation (1) (thick continuous line) which conformed with Trevan's observations, though the latter involved actual values of the viscosity which were about 1.7 times our values for blood of the same concentration. Since these higher estimates of the viscosity were due to the low pressure driving the blood in the Ostwald type of viscometer, it would appear that the shape of the curve is independent of the pressure.

Within the limits of corpuscular concentration of 10 to 80 p.c. equation (1) may be modified to

$$\eta = \frac{k\eta_0}{1 - \phi^{\frac{1}{3}}}, \quad \dots\dots(2)$$

where η is the apparent viscosity of blood of corpuscular concentration ϕ , the plasma having a viscosity η_0 . k is a constant increasing with pressure if the pressure is low, but approximately equal to 0.6 over a wide range of higher pressures, such as obtain in any high velocity type of viscometer. Trevan's viscometer happened to employ a pressure low enough to increase k to 1.0. Still lower pressures would increase it further. The interest of these observations is in the independence of the shape of the curve, as plotted in Fig. 11, of the velocity of the blood, although the actual viscosity is dependent on it. Equation (1) was derived by Hatschek [1911] in connection with a system altogether different from blood, and can only be adopted provisionally as an approximate empirical relation in this connection.

The values of the viscosity obtained in the hindlimb, also plotted in Fig. 11, evidently are related to the corpuscular concentration in a markedly different fashion, and this is presumably due to the reduction in the effective bore of the tubes. The divergence between these particular relations in large and small tubes appears to be greater for low than for high concentration of corpuscles, contrasting in this respect with the difference in apparent viscosity itself, in which the effect is increased at high corpuscular concentration (Fig. 10). If the anomalies of blood viscosity are due to the occurrence of a cell-free zone in the peripheral part of the tube, one might perhaps expect that the effect of reducing the bore of the tube would be less well marked at high corpuscular concentrations, and it is possible that this would account for the closer correspondence between the curves of Fig. 11 at higher than lower concentrations.

Two inferences from the curves of Fig. 11 may be applied to most practical purposes: (1) The ratios of blood viscosities at different corpuscular concentrations are likely to be the same in all viscometers, since no viscometer in common use has a bore small enough to affect the relation. Therefore the contention of the advocates of the high velocity of type viscometer, that the innumerable observations with the Ostwald type of viscometer are meaningless, cannot be maintained when the observations are concerned with this relation. (2) Though the actual values of the apparent viscosity of blood are widely different in the limb and in a viscometer, the ratio of the viscosity of an abnormally concentrated blood to that of normal blood is not very different in the two, and viscometer readings yield a fair indication of the increased resistance to circulation in a condition of polycythæmia. The deviation of the viscosity of anæmic blood from that of normal blood is, however, so different in the limb and the viscometer, that no fair estimate can be derived from viscometer readings; in this respect the Ostwald type of viscometer is no more misleading than a high velocity type.

SUMMARY.

1. The value of the apparent viscosity of defibrinated blood flowing in the vascular bed was determined experimentally. This could not be predicted from observations with glass viscometers, since a change in design may produce a fiftyfold variation of the apparent viscosity of a given sample of blood.

2. In justification of the choice of the isolated hindlimb of the dog as a biological viscometer, its hydrodynamic and hæmodynamic properties are contrasted with those of the kidney (Fig. 1).

3. Pressure-flow curves in the limb are approximately linear both for Ringer (Fig. 2) and blood (Fig. 6), except at low pressures. The values at which the lines intersect the pressure axis depend on the back pressure due to œdema in Ringer perfusion, and on the anomalous viscosity of blood in blood perfusion; the intercept increases with increase of corpuscular concentration (Fig. 6).

4. Perfusion of a limb with blood from a heart-lung circulation, interrupted by short periods of Ringer perfusion, enables the Ringer flow to be compared with the blood flow (Fig. 3) uncomplicated by œdema. In such experiments the ratio of the blood flow to the Ringer flow gives an apparent viscosity of normal defibrinated blood of 2.2 ± 0.2 at 90 mm. Hg, *i.e.* about one-half the value given by a glass viscometer.

The intercept of the blood pressure-flow curve exceeds that of the Ringer curve by 17 ± 3 mm. Hg.

5. The effect of corpuscular concentration on the apparent viscosity of blood in the limb was studied in the triple pump-lung limb preparation (Fig. 4), in which the concentration could be varied within the range of 2-80 p.c. without the bore of the arteries changing (Fig. 5). In stable preparations the rate of flow of normal blood, tested before and after each excursion to perfusion with abnormal blood, is constant within 2 p.c. throughout the experiment.

6. Assuming the plasma viscosity to be the same in the limb and the glass viscometer, the apparent viscosity at 90 mm. Hg pressure of dog's defibrinated blood of normal concentration (48 p.c.) so measured is 2.2 ± 0.2 in the limb; it is 4.4 ± 0.3 in the high velocity type of glass viscometer which gives lower values than the low velocity (Ostwald) types. The relations between viscosity and corpuscular concentration measured both in the limb and the glass viscometer are recorded in Fig. 8.

7. Change in arterial pressure produces little change in the apparent viscosity of normal blood. If the pressure falls below 50 mm. Hg the viscosity increases slightly. At higher corpuscular concentrations the increase of viscosity with reduction of arterial pressure is greater (Table III).

8. The low value of the apparent viscosity in the limb is attributed to the small diameter of the blood vessels in which most of the arterio-venous pressure fall occurs. Certain properties of the physical mechanism involved in the anomalous viscosity of blood are described (Figs. 10 and 11).

We have pleasure in expressing our indebtedness to Dr L. E. Bayliss for his help and advice at many stages of the enquiry, and particularly for his permission to quote those of his unpublished observations which throw light on ours.

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THE ADRENALS AND ANÆSTHETIC HYPERGLYCÆMIA.

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THE object of this work was to indicate the rôle of the adrenal glands in increasing the blood sugar p.c. (glycæmic response) during anæsthesia. Elliott [1912] stated that all the ordinary conditions of anæsthesia with ether, chloroform, and urethane were attended by exhaustion of adrenaline. Rogoff and Stewart [1920] from a large series of experiments and Burn [1915] from adrenalectomy experiments during anæsthesia claimed that the hyperglycæmia associated with anæsthetization in cats was not dependent on the adrenals. Macleod [1926] wrote in favour and Evans, Tsai and Young [1931] cited four experiments indicating their connection in the course of their work on liver glycogen. A general objection, however, can be taken to conclusions based entirely on the results of operations on the adrenals, the adrenal medullas or their nerve supply on the ground that the animals so treated were not normal or were largely incapable of showing the usual reactions of intact animals to anæsthetics.

METHODS.

Three methods were used, operative, indirect and comparative, to determine the glycæmic response to anæsthetics in rabbits fed on the standard laboratory diet. The open method of anæsthetization was preferred. The aim was to select rabbits which took the anæsthetic well, and those animals were discarded which resisted and struggled during one or two trial inductions.

Operative method.

A series of thirty rabbits of about 1.5 kg. was prepared by two-stage aseptic operations under open etherization which aimed at decreasing or immobilizing the available adrenaline by double adrenalectomy (ten), unilateral adrenalectomy with contra-lateral medulliadrenalectomy or

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denervation and decapsulation (ten), and double medulliadrenalectomy or double denervation and decapsulation (ten). These three groups of rabbits have been designated A.A., A.M. and M.M. respectively. The interval between operations was not less than 14 days.

The glands were exposed through the lumbar extra-peritoneal route and removed after ligation of the blood vessels.

Medulliadrenalectomy was carried out by curetting or cauterizing the medulla after puncturing the gland with the scalpel point. Curettage was ultimately the method of choice, as in one or two cases the cortex had apparently been damaged by heat—as judged by the after-history of the animals. From the beginning of etherization to skin suturing occupied about 30 min. as a rule. There was no sepsis in the series. The essential point in post-operative treatment was to keep the animals warm, but precautions against exposure to cold were necessary only for a few weeks at night during the cool season in India.

The glycaemic response to open etherization was determined during the post-absorptive period (16 hours' fast) by repeated tests at intervals of several days (4 or 5) before operation. Blood samples for sugar estimation [Hagedorn and Jensen, 1923] were taken from the marginal ear vein before etherization, after induction (5 to 7 min.) and at end of 30 min. surgical anæsthesia. Blood-sugar records for the operations were also made.

Weight records were kept throughout and blood concentration was studied by specific gravity readings (chloroform and benzene method), r.b.c. counts and Hb p.c. determinations (colorimetric method). Notes on the cardiac action and rate were made, as determined by palpation during the experimental anæsthesia.

Results (Fig. 1). During surgical etherization for 30 min. the increase in blood sugar was invariably greatest in the intact rabbit, slightly less after the first and least after the second operation.

The glycaemic response of those A.A. rabbits with signs of adrenal insufficiency was noticeably small, and very small 2 or 3 days before death.

The glycaemic response of the A.A. rabbits which survived for long periods in good health was slightly less than for the A.M. and M.M. groups.

The fasting blood-sugar level was low (40–60 mg./100 g.) in those rabbits with signs of adrenal insufficiency.

In the remaining groups of double operated rabbits the average fasting blood-sugar level was about 85–90 mg./100 g. as compared with 105–115 mg./100 g. for the intact animals.

Further differentiation of the A.M. and M.M. groups was not justified, as the difference in the average fasting levels of intact and single operated rabbits was small and less than the "probable error" obtained on statistical investigation.

In the A.A. rabbits which died of adrenal insufficiency the loss of weight was progressive and averaged 20 p.c. at death. In the other

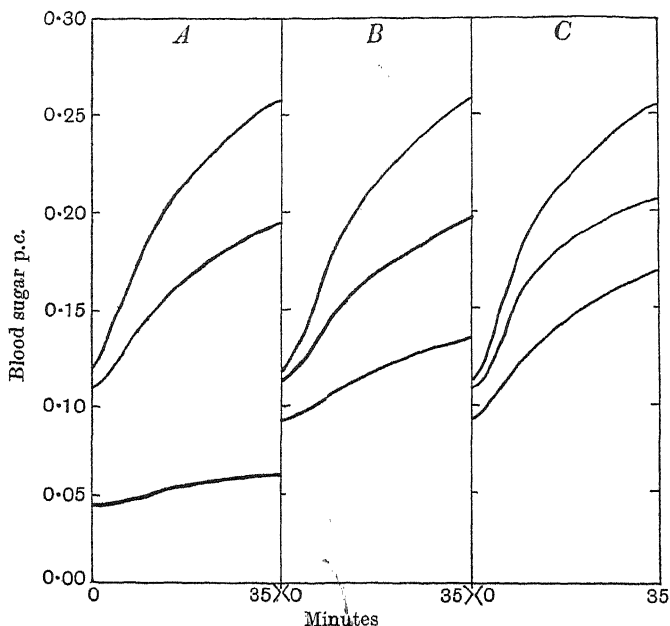


Fig. 1. Effect of ether anaesthesia on blood sugar. Ether begun at 0 min. Uppermost lines in A, B, C are for intact, middle for single operated and lowest for double operated rabbits. A = A.A. rabbits which developed signs of adrenal insufficiency. Each graph is mean of six experiments on three rabbits. B = A.A. rabbits which survived without signs of adrenal insufficiency. Mean of fourteen experiments on seven rabbits. C = A.M. and M.M. rabbits. Mean of forty experiments on twenty rabbits.

groups the weight decreased slightly after the operations but remained more or less steady thereafter.

Discussion. Some but not all A.A. rabbits showed signs of adrenal insufficiency. This was clear from results in a series of thirty rabbits adrenalectomized by the above two-stage method and retained for observations summarized elsewhere [Reid, 1932]. Three died within a day or two after the second operation, probably from extrinsic causes, nine died between the 9th and 17th days, and eighteen were living more

than 30 days after the second operation, apparently in good health. Therefore 60 p.c., or if we exclude deaths within a day or two, 66 p.c. survived for long periods. This result may be correlated with Kojima's [1929] detection of accessory adrenal tissues in 70 p.c. of normal rabbits, although the number of our rabbits (thirty) is small.

Adrenal insufficiency was shown by a steady fall in weight, hæmo-concentration, slow and feeble heart action during etherization and low blood-sugar level. Four rabbits from the operated groups were killed for liver glycogen estimation. It seemed from these few results that continued fall in body weight after the second operation indicated low liver glycogen. Muscle glycogen was not studied. Weight records were relied upon as an index of the general well-being of the operated animals.

Results got from rabbits which showed early or, after a few days, obvious signs of adrenal insufficiency are not admissible owing to the presence of disturbed general or carbohydrate metabolism, indicated by the decreasing body weight and fasting blood-sugar level. As M.M. rabbits survived for long periods apparently in normal health, it seems that the adrenal cortex is concerned with general and, indirectly at any rate, with carbohydrate metabolism.

Results are admissible from A.A. rabbits surviving for long periods apparently in good health, but the possible influence of decreasing the available cortical tissue by operation must be considered. This point is met by results from M.M. experiments. But the decreased glycæmic response of these rabbits might still be due partly to decrease of those circulatory and other adjustments normally seen in anæsthetized intact rabbits.

In the A.A. rabbits with signs of adrenal insufficiency the heart rate during anæsthesia was easily counted on palpation, and was much less than for the normal anæsthetized animals. Our data from the other double-operated rabbits indicated some decrease as compared with the normal during anæsthesia. If we postulated even a moderate decrease, this might explain partly the decreased glycæmic response. Accurate data were not available, in our form of experiments, on the effect of variations in the general and liver circulations of intact animals in increasing the blood sugar during a period of active glycogenolysis. In the course of later experiments on dogs the increases in blood sugar p.c. and pulse rate were found to be approximately contemporaneous during and after anæsthetization with ether or chloroform.

With the usual anæsthetic dose of amytal, on the other hand, blood sugar p.c. and pulse rate remained more or less steady, and Murphy

and Young [1932] found least fall in liver glycogen with amytal of the anæsthetics tried. Another factor that occurred to us as having some relation to the circulation through the liver was the respiratory rate. The action of the respiratory muscles appeared weak in A.A. rabbits with slight signs of adrenal insufficiency, but not in the other groups. The relation of this to the decreased glycæmic response in the former is doubtful. In one dog the resting respiratory rhythm was halved during amytalization and doubled during etherization. It would be difficult to determine what effect variations in respiratory rhythm had on the liver circulation in the intact animal and consequently *per se* on blood sugar p.c. under different experimental conditions.

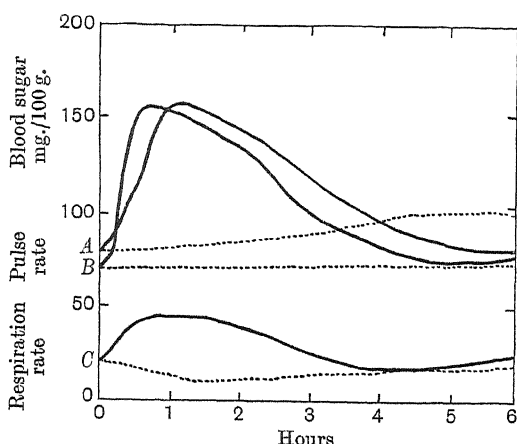


Fig. 2. Effect of ether (solid lines) and amytal (broken lines) on blood sugar (mg./100 g.) (A), pulse rate per min. (B), and respiration rate per min. (C). Etherization, begun at 0 min., lasted 1.5 hr. Amytal at 0 min. Mean of eight experiments on dogs.

It should not be understood that the above is put forward here as accounting for the different effects of ether and amytal, but as an indication of factors to be considered in assessing the results of anæsthetics on blood sugar p.c. in intact and operated animals.

In cases of interference with medulliadrenal function Britton, Calvery and Geiling [1928] have shown that the complete suppression of adrenaline secretion by evacuation of the medulla in cats resulted in a marked increase in sensitivity to insulin injection, although the hepatic glycogen reserves were maintained at practically normal levels. Such increased sensitivity to insulin offers a possible explanation, but perhaps not a likely one, of the decreased glycæmic response to anæsthetics

obtained in the operated groups, if an increased insulinæmia is postulated as occurring during anæsthesia or in association with the rise in blood sugar. In view of the above considerations further tests were made on intact animals in the indirect and comparative methods.

A preliminary conclusion is made that the adrenals play a part in producing anæsthetic hyperglycæmia.

Indirect method.

The indirect method consisted in using combinations of anæsthetics or narcotics. It comprised the study of the glycæmic response of normal and amytaled animals to (1) ether, (2) morphine narcosis, (3) adrenaline. Most of the experiments under (1) were done on dogs. Chloroform was also used instead of ether in both rabbits and dogs.

(1) The concentration of ether and optimal respiratory rate necessary to maintain surgical anæsthesia by the "Ideal" respiration pump were determined for normal tracheotomized animals of nearly the same weight as those used for the remaining experiments. Dogs were nearly 10 kg. and rabbits 1.5 kg.

The glycæmic response to ether was determined during 2 hr. surgical anæsthesia in eight dogs and two rabbits by repeated tests. After a week's interval amytaled (B.D.H. Ltd.) was injected intraperitoneally (dog 60 mg. per kg.; rabbit 50–80 mg. per kg.). After anæsthetization in 10–30 min. tracheotomy was done and ether administered for 2 hr.

(2) The minimal subcutaneous dose of morphine sulphate to produce in 1 hour an increase of at least 100 mg./100 g. in the blood sugar was decided for each rabbit. This varied between 10–20 mg. per kg. for different animals in the series used. After the usual interval the rabbit was anæsthetized by amytaled and morphine given in the requisite dose for that animal. Tracheotomy and artificial respiration by pump were begun at the earliest sign of depression of the respiratory centre, if necessary. In some cases artificial respiration by hand was sufficient.

(3) Adrenaline (Parke, Davis & Co.) was used subcutaneously (0.15 mg. per kg.) in rabbits.

The alkali reserve of the blood was determined in sixteen dogs during ether chloroform and amytaled anæsthesia by van Slyke's method [1922].

Results (Figs. 3 and 4). Amytaled inhibits partly the hyperglycæmia occurring normally in ether or chloroform anæsthesia in rabbits and dogs.

Amytaled checked the normal glycæmic response to morphine more than that to ether or chloroform.

Amytaled did not prevent adrenaline hyperglycæmia of the normal order.

The alkali reserve was decreased during ether or chloroform anaesthesia but not with amytal.

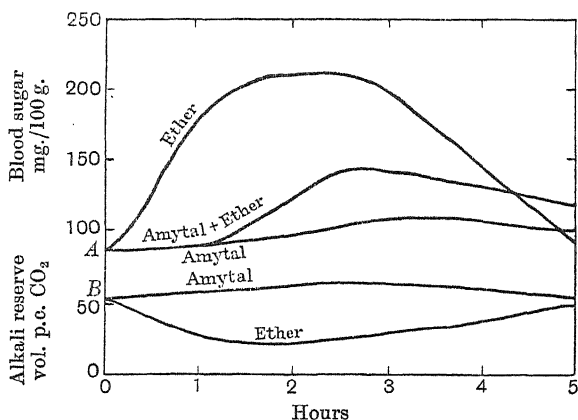


Fig. 3. *A*, Effect of ether, amytal and ether, amytal on blood sugar (mg./100 g.). Mean of eight experiments on dogs. Ether at 0 hr. for 2 hr. Amytal at 0 hr. and ether at 1 hr. for 2 hr. Amytal for 0 hr. *B*, Alkali reserve of blood (vol. CO₂ p.c.). Mean of eight experiments.

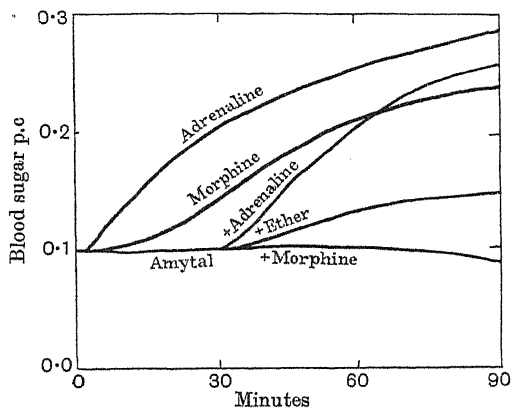


Fig. 4. Effects of adrenaline, morphine, amytal and adrenaline, amytal and ether, amytal and morphine on blood sugar. Adrenaline, morphine at 0 min., amytal at 0 min., followed by adrenaline and ether, morphine at 30 min. Mean of eight experiments on rabbits.

Discussion. Rogoff and Stewart [1922] admitted that the adrenals were directly concerned in morphine hyperglycæmia (confirmed by us) and were unable to correlate the size of the increase in blood sugar p.c. with the liver glycogen content of their intact and surviving adrenal-

ectomized animals. It is clear from our results that an amytalized rabbit showed adrenaline hyperglycæmia of the usual order. Since the glycæmic response to morphine was of negligible amount in the amytalized animal, it seemed probable that amytal inhibited the usual adrenalinæmia after morphine and, *a priori*, with ether or chloroform anæsthesia. The small decrease in liver glycogen in the course of 10–30 min. after amytal in anæsthetic dose [Evans, Tsai and Young, 1931] could not account for the markedly decreased glycæmic response to ether or chloroform.

As regards evidence of the presence of adrenaline in the blood the effect of serum from anæsthetized dogs was tried on rabbits' intestine suspended in a modified Tyrode solution in the Burn-Dale bath. Buckmaster and Gardner [1910] found that the concentration of chloroform in the blood of dogs was 0.03–0.04 p.c. after 1 hour's anæsthesia. To a known volume of bathing fluid was added a fixed amount of (a) normal dog's serum, (b) normal dog's serum plus 0.03 p.c. chloroform, (c) amytalized dog's serum, (d) anæsthetized (chloroform) dog's serum. The bathing fluid was changed after each addition of (a), (b), (c) or (d). Several experiments were done, the additions of serum not always being made in the same order. The slight definite decrease of tone and of the intestinal contractions was always more marked for (d) than for (a), (b) or (c). This result was suggestive, although the addition of dog's serum to rabbit's intestine introduced complicating factors.

Apart from their effects on blood sugar p.c., morphine and amytal differed in that the former caused a rise in body temperature of 1°–2° F. in many rabbits in the course of an hour, while the body of an amytalized rabbit under similar conditions (not on a hot plate) tended to cool.

Morphine hyperglycæmia was obtained in intact rabbits without very deep narcosis and without the struggling and disturbed respiration often associated with the induction period of ether. This suggests that hyperglycæmia could occur in anæsthesia probably through an adrenalinæmia not necessarily associated with the concomitant struggling and disturbance of respiration of the induction period.

Correlation between the rise of body temperature and blood sugar was lacking in the rabbits examined.

Comparative method.

Rabbits of approximately the same weight were given 0.15 mg. adrenaline per kg. body weight in the post-absorptive state and the glycæmic response noted at 10 and 60 min. If the increase in blood sugar was small, the same amount of adrenaline was injected after several days

in divided doses at different sites to eliminate the possibility of variation in the absorption rate. Two groups of rabbits were made, one showing a well-marked rise of at least 150 mg./100 g. and another showing a comparatively small rise of less than 100 mg./100 g. in blood sugar. Their blood-sugar increase to etherization was determined according to the usual routine.

Result (Fig. 5). The anæsthetic glycaemic response was greater in the group of rabbits which had shown previously a large increase in blood sugar in response to adrenaline than in the group less sensitive to adrenaline.

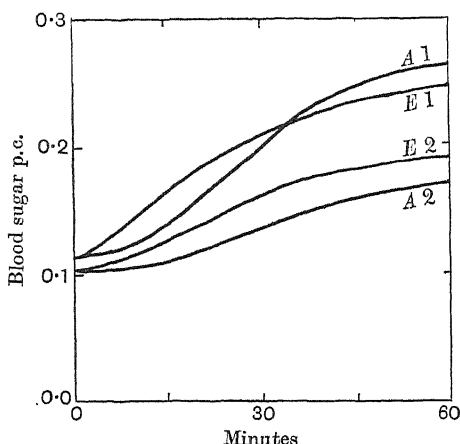


Fig. 5. Effect of ether (*E 1*; *E 2*) at 0 min. on blood sugar of two groups of rabbits which showed large and small adrenaline hyperglycæmias (*A 1*; *A 2* respectively). Each graph is the mean of six experiments.

Discussion. The above result is taken as confirming that the adrenals take part in increasing blood sugar during surgical anaesthesia. Bose [1930], quoting the results of experiments by Acton and Bose on the alleged deterioration of insulin in India, stated that in rabbits of the same species but of different colours adrenaline caused a much larger increase in the blood sugar of the albino variety (Himalayan) than in either the black (Himalayan) or brown (Belgian hare) variety. The average increase in blood sugar in 1 hour after 0.15 mg. adrenaline was 146 mg./100 g. for ten albinos and 41 mg./100 g. for six Belgian hare rabbits. The actual increases in mg./100 g. noted by us were 88, 145, 123, 167, 66, 147 (mean 123) for six albinos and 57, 80, 143, 147, 58, 106 (mean 102) for six Belgian hare rabbits. The rabbits were chosen at random from the two species. This result suggests not that the sensitivity

of rabbits to adrenaline could be gauged by their colour, but that, in studying the relationship of the adrenals to experimental hyperglycæmias, clearer results would emerge from using animals proved sensitive to adrenaline injection from the size of the blood-sugar increase.

SUMMARY.

1. The part played by the adrenal glands in increasing the blood sugar during 30 min. surgical anæsthesia has been investigated by three methods, operative, indirect and comparative. Rabbits mainly and, for some observations, dogs were used.

2. The anæsthetic glycæmic response of operated rabbits was less than for intact animals and markedly so for the double operated animals.

3. Points for consideration in regard to conclusions based only on results from operative methods have been indicated.

4. In the indirect method combinations of anæsthetics were used, *e.g.* amytal and morphine, amytal and ether or chloroform.

5. Amytal inhibited the usual hyperglycæmia occurring in ether or chloroform anæsthesia and in morphine narcosis, but not following subcutaneous injection of adrenaline.

6. In the comparative method the blood-sugar increase of anæsthetized rabbits sensitive to adrenaline has been compared with that for rabbits less sensitive and found to be greater.

7. The advisability of using animals sensitive to adrenaline in studies on the relationship of the adrenals to experimental hyperglycæmia has been indicated.

8. It is concluded that the adrenals are concerned partly in increasing the blood sugar during 30 min. surgical anæsthesia with ether or chloroform.

Part of the expenses of the investigation was paid for by the Indian Research Fund Association during 1930-2. We desire to express our thanks for the grants allotted towards the expenses and the salaries of a research assistant and laboratory technician.

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ALPHA AND GAMMA CURVES IN SLOW MUSCLES.

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FOLLOWING the discussion which has taken place in this *Journal* [Rushton, 1930, 1931, 1932 *a, b, c*; Lapique, 1931 *a, b*, 1932] regarding the significance of Keith Lucas's α curve, I have taken up again the experimental study of this phenomenon in slow muscles.

LONGITUDINAL MUSCLE OF *HOLOTHURIA TUBULOSA*.

Holothuria provides on the internal side of its somatic wall five longitudinal strips of muscle. These strips can be seen distinctly on opening the general cavity; they are long and composed of parallel fibres. This disposition has already been utilized in physiology, especially by A. V. Hill [1926], and by G. Morin [1931]; it seems to present the anatomical conditions allowing a free development of the α effect.

We have repeated on this muscle the experiments made on the frog's sartorius. These experiments were performed in the Marine Biological Laboratory of Tamaris last September, where we found the appropriate equipment very easily, as Prof. Cardot, whom we have the honour of counting among our former students, uses our methods habitually; thus it is unnecessary to insist on the details of the technique.

The only particular point relates to the muscle itself, which is in a state of contracture after the dissection. Remembering that in our experiments on the Snail, chloroform gave us good results in case of contracture [Lapicque et Laugier, 1927], we used this same procedure with the *Holothuria*. When placed in a bath composed of nine parts of sea water and one part of the same saturated with chloroform, the animal shows at first a stimulation phase during which it may eject its entire intestinal tube (a frequent phenomenon with *Holothuria* under any kind of serious disturbance, asphyxia for example); then it stretches out and remains still and completely relaxed. But the dissection again causes, especially in the muscle considered, a contracture which does not seem to differ in any way from the normal one. Therefore we gave up this method, which otherwise does not seem to have any effect on the phenomena we are studying. Moreover, according to the

general rule for smooth muscle, and as has been observed for this particular one by A. V. Hill and others, the muscle gradually relaxes. Thus, after dissection, the muscle was placed under a layer of a few millimetres of sea water at the bottom of a glass rectangular trough and fixed at its two ends to two light vertical levers slightly stretching it, and adjustable as the muscle extended. A contraction produced an oscillation of a lever; the threshold was detected by the smallest oscillation perceptible to the eye, the upper edge of the lever being projected usually on a scale, for we know that the direct observation of such slow muscles give thresholds which are very hard to perceive.

Under a stimulation of one or several hundredths of a second and of a notably higher intensity than the threshold, the course of the lever could be followed by the eye with a stop-watch and the duration of the contraction thus appreciated without any graphs. On one of our muscles we have noted in this manner that the total contraction, homologous to the twitch of the rapid muscles, lasted 6–8 sec. of which 2 to 3 corresponded to the shortening phase.

The temperature was relatively high, around 28° C., in the room; this was also the outside temperature in the shade with very small variations; the sea water used had always been left in the room long enough to reach the temperature equilibrium. But owing to this high temperature all the time elements in this series of experiments must be considered as accelerated when compared to other measurements made at "ordinary temperature." Thus on Hill's graphs of the muscle contraction of *Holothuria nigra* at Plymouth we read a duration for the ascending phase of about 5 sec. for a total duration of about 40 sec. This total duration cannot be compared to ours, for the stimulation was produced by the break shock of an induction coil; it is known that such a stimulation, very short in regard to the time relations of the muscle, slows down relaxation; in fact the ascending phase constitutes only one-eighth of the total duration instead of one-third or one-fourth as in the normal twitch. But the duration of the ascending phase, which is not affected by this inappropriate form of stimulus, is about twice what we observed. I am inclined to consider this difference as due to the difference of temperature, about 10° C., rather than to the difference of species.

On the other hand, the chronaxie measured with our usual technique (stigmatic cathode on the muscle in the air, determination of the liminal voltage for long durations, then liminal duration for a voltage twice the preceding one) was 9σ on the same muscle with which we measured the contraction duration. Some following specimens gave us slightly smaller chronaxies as low as 5σ . Morin had found an average of about 20σ ; I was told by Prof. Cardot that his experiments had been made in the same laboratory but at the beginning of April. The temperature was certainly much cooler and the difference, which is about the same as for the contraction duration, can, very likely, be explained in the same way.

Let us make a comparison with the skeletal muscles of the frog; we can take for these a chronaxie of 0.3σ as an average at ordinary temperature and a twitch duration of one- to two-tenths of a second with

an ascending phase about 0.05 sec. Thus we can say that the *Holothuria* muscle is about 40 times slower so far as contractility is concerned. The chronaxie shows also a lengthening in relation with this slowness.

Now we must find out what can be obtained with such a slow muscle when studied in an electrolytic solution which diffuses the current lengthwise over a great length, that is to say in the conditions which, for rapid muscles, give strength-duration curves called α and γ .

First we used the technique which, on the frog sartorius, allowed us to separate almost completely and at will the α and γ curves, that is to say, through the physiological solution (here sea water) a silver wire touching the muscle (stigmatic electrode) and a larger silver electrode dipped in the bath at some distance on the axis of the muscle (diffused electrode). When the cathode is constituted by the stigmatic electrode we get a γ curve; when it is diffused we get chiefly an α curve, with often small portions of γ curve for short durations.

It should be remembered that the time constant of γ thus obtained in the bath is much smaller than that of α , but markedly longer (two to three times) than the chronaxie measured in the air.

Our first similar trial on the *Holothuria* muscle gave us a totally opposite result, that is to say, no difference between what should have been α and what should have been γ ; and furthermore no difference with stimulation in the air.

Exp. of Sept. 11, 26° C. Holothuria caught in the morning, dissected at 2 p.m.; muscle left to rest 1 hour in sea water. One end of the muscle raised above the water so as to be stimulated in air; the simple chronaxie measurement gives 10σ . When the whole preparation is dipped in sea water, the chronaxie is again 10σ . Then the strength-duration relation is determined, first with a stigmatic cathode, then with a diffused cathode. For each tested duration between 100 and 10σ the liminal potentials were the same, in either one or the other methods, within experimental error.

Therefore, we doubted if the current was really reversed, but, verifying the circuit we noticed that the second series of figures was really obtained with a diffuse cathode; then we reversed the current again and found again a series of values which differed from the preceding ones only by a slight rise of the rheobase, a difference without significance.

These are the figures obtained (durations in σ , liminal potentials in arbitrary units):

Duration	Stigmatic electrode		
	Cathode	Anode	Cathode
∞	8	9	10
100	9	9.5	10
40	10	10	11.5
30	11	11	—
20	12.5	13	14
15	15	15	16
10	19	19	19.5
100	9	—	10

The stigmatic electrode is placed closer to the end opposite to the diffused electrode; the simple measure of chronaxie gives 10σ with either direction of current. When we return the electrode to the opposite end, the chronaxie is 11σ for either direction.

In the following experiment the two curves present but a very slight difference.

Sept. 12, 28° C. *Holothuria* previously chloroformed. The time required to obtain a good relaxation of the muscle is as long as the day before when we operated without chloroform. Stigmatic electrode near the end of the muscle toward the diffused electrode.

These are the liminal voltages in the experimental order:

Durations	Stigmatic anode	Stigmatic cathode
∞	6.0	6.4
100	6.3	6.4
7	16	—
40	7.0	7.0
30	7.5	—
20	8.5	8.5
10	12	11
7	—	14
100	6.0	—

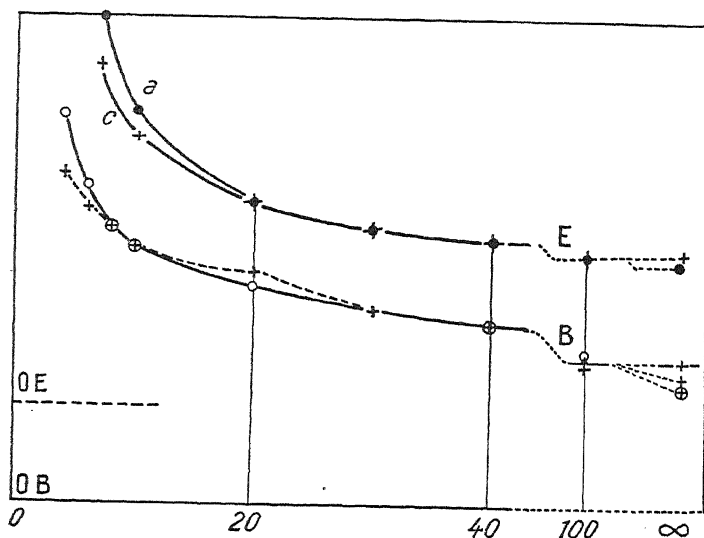


Fig. 1. Strength-duration curves from the *Holothuria* muscle: B, under a block; E, with a stigmatic anode (a) or cathode (c).

Between 100 and 20σ the figures are practically identical; below 20σ the threshold rises perhaps a little slower with the stigmatic cathode (Fig. 1 E).

A series of simple chronaxie measurements, with stigmatic electrode moved from place to place, or with the diffuse electrode near the other end of the muscle, gave values always about 9σ for either direction of the current, a value quite similar to the one obtained by interpolation in the above series.

Then we applied to this muscle Rushton's block device: thickness of the block, 20 mm.; cathode either on the right or on the left.

The liminal voltage for a duration which can be considered as infinite (closing of the circuit by hand for about 1 sec.) is always a little higher in the first case (6.5–7.5) than in the second (5–6), but we did not regularly find the same values again when we made our measurements alternatively in one direction and then in the other. The liminal durations corresponding to twice these values vary (between 6 and 10σ). The study of the strength-duration curves shows that the source of difficulty for the chronaxie measurements lies in the uncertainty of the liminal voltage for long durations which should be, but is not always, a rheobase. The figures corresponding to durations less than 0, 1 sec. are sufficiently constant. The following figures give the values obtained successively with the cathode on the right of the block (in each pair of figures, the first represents the durations of current flow in σ ; the second, the liminal voltage in arbitrary units):

∞ , 7.5; 100, 7.5; 40, 9.5; ∞ , 7.5; 20, 12; 10, 13–13.5; 8, 14.5; 6, 16; ∞ , 6.5; 100, 7.5; 30, 10.3; 20, 12; ∞ , 6.8; 100, 7.2; 6, 15.5; 4, 17.5.

For the cathode on the left, we obtain:

∞ , 5.0; 100, 6.3; 40, 7.5; 20, 9.0; 10, 10.5; 8, 11.5; 6, 13; 4, 16; 100, 6.3.

If we recalculate the values of the first series in order that the two curves coincide for 100σ and if we plot these figures we get the graph 1B which shows as 1E two very similar curves.

Other specimens which had a smaller chronaxie have given two curves showing a greater difference; but nevertheless the chronological difference between these two curves is always very small compared to the difference between α and γ of the frog muscle.

In the following experiment a muscle with a chronaxie of 5σ gave an opportunity for a more complete study.

Sept. 13. Large *Holothuria* chloroformed in the morning for the dissection; the muscle was preserved in sea water without chloroform; after a few trials in the morning showing a varying excitability, the muscle

became stable and gave us a series of results which can be summarized as follows:

I. Under a 20 mm. and a 30 mm. block the curves are simple and can be superimposed, their pseudochronaxie being between 13 and 16 σ .

II. With a stigmatic cathode through the solution the curve is simple with a pseudochronaxie of 8 σ .

III. With a stigmatic electrode in the air the curve is simple, as expected, and the chronaxie is about 5 σ .

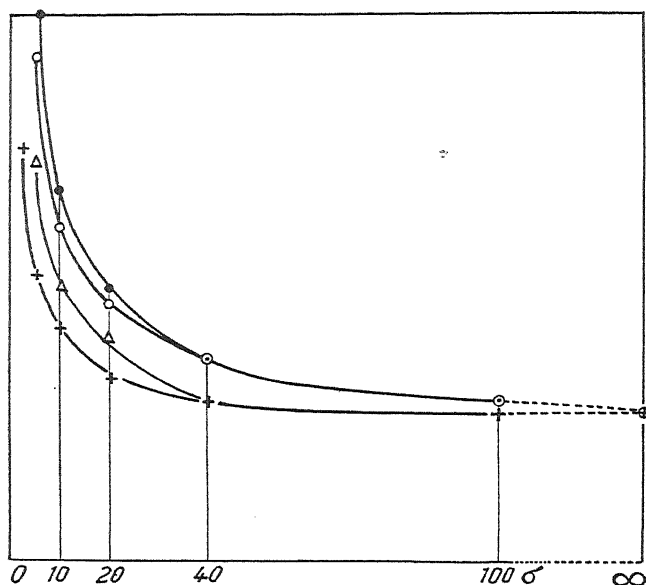


Fig. 2. Strength-duration curves from the *Holothuria* muscle, according to varying conditions (see text).

The following table gives the figures which have been plotted on Fig. 2 after having been equalized for infinite durations.

Durations	Block		Stigmatic cathode		
	20 mm.	30 mm.	In the solution	In the air	
				(1)	(2)
∞	2.9	4.0	5.8	1.0	2.2
100	3.3	4.5	6.1	1.0	2.2
40	4.1	5.5	6.5	1.1	2.4
20	5.4	7.0	9	1.2	2.6
10	7.5	9	11	1.4	3
5	11	14	16	1.9	4.3
3	—	—	—	2.8	—

The experiment with the stigmatic electrode in the air was done twice, first at the beginning about noon, then toward the end about 5 p.m. The two curves can be superimposed (except for one figure which is undoubtedly erroneous) and the chronaxie has remained the same. So that the different curves actually represent the influence of the conditions and not a progressive change in the muscle excitability.

These different conditions give a set of curves offering differences in the same sense as for the frog, but relatively very small.

The blocks should give α curves, the stigmatic cathode in the solution a γ curve; in fact the time constant is longer in the first case than in the second, but only doubled instead of 10 or 20 times longer.

The treatment with chloroform previous to the dissection has nothing to do with these results, as their essential character was found again the next day on a non-chloroformed animal.

Sept. 14. (Third experiment.) *Holothuria* dissected at 3 p.m. without chloroform. Readings begun at 5 p.m.

I, 20 mm. block; II, stigmatic cathode in the air.

Durations	I	II
∞	4	2.8
100	4.4	2.8
40	6.0	3.0
20	7.5	3.6
10	9.0	4.5
5	12.0	5.6
4	—	7.0
40	6.0	—

If we consider the durations below 40σ , we can see that if we double the values of II they become equal to the corresponding values of I within the range of experimental error. I had already noticed this fact in the course of the experiment, and supposing that the deviations for the longer durations in the curve I came from a δ curve [Lapicque, 1931 b] I had concluded that the curves could be superimposed.

I had still this idea when I wrote the Note for the Société de Biologie [Lapicque, L. et M., 1932]. But later, plotting the graph on a sufficiently extended time scale, I noticed that the curve I is perfectly smooth; therefore we must compare I and II after they have been equalized for infinite duration. We then get the curves *A* and *B* of Fig. 3. When made equal for 40σ , the curve *B* is replaced by *B'*, which shows that the differentiation between these two curves is slight enough to have been overlooked.

Whatever the means of stimulation used, none of our experiments on *Holothuria* muscle gave any curves more different nor any distinct kink in the curves. It is easy to find among previously published experiments on the frog sartorius some α curves obtained on one and the same muscle as different and even more different one from another, though all of them very distinct from γ .

Let us now see what is obtained on the *Holothuria* muscle with electrodes of varying diameter.

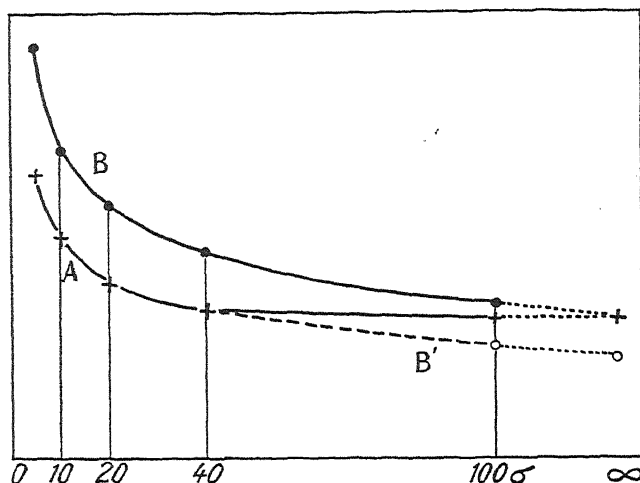


Fig. 3. Strength-duration curves from the *Holothuria* muscle: A, with a dry stigmatic cathode; B or B', under a block.

We improvised at the station of Tamaris a series of electrodes with endings similar to those described in the preceding paper [Lapicque, 1932, p. 265]. The following figures give the diameters in millimetres: A, 0.8; B, 0.4; C, 1.8; D, 2.8; E, 1.1; F, 2.2.

For this series of diameters the simple measure of chronaxie (liminal duration for an intensity twice that of the rheobase) shows no conspicuous influence of this factor. The chronaxie appears to be practically independent of the size of the electrodes.

The following table gives two series of measurements in σ :

I: A, 5-6; B, 4.5-5; C, 4.5; D, 4.5.

II: A, 5; B, 5; E, 5.5; F, 6; C, 6; B (control), 5.

On the second series the diameter increasing from 1 to 5 or 6, the chronaxie increases from 1 to 1.2. This increase seems to be systematic,

but its magnitude is within the range of experimental errors. And moreover the series I, where the diameter varies from 1 to 7, shows no systematic variation of chronaxie.

MUSCLE RETRACTOR OF THE HEAD OF *TESTUDO MAURITANICA*.

I looked for a muscle which, as far as the rapidity is concerned, falls between the one of the *Holothuria* and that of the frog. I thought of the terrestrial turtle (the species found commercially in Paris is *Testudo mauritanica* brought from Algeria). I had previously noted on the muscles of the limbs in relation with the slowness of contraction, chronaxies 5 to 10 times longer than in the frog [L. et M. Lapique, 1927]. Already in 1926 Hartree had used this slowness to analyse more conveniently the phases of the heat production during the contraction; he estimates that the series of events in the biceps cruris of this animal takes place in a time 15 times longer than in the sartorius of the frog.

After a few trials on different leg muscles I chose the retractor of the head; this muscle is particularly adapted for this kind of experiment on account of its regularly prismatic disposition and of its length, which, when extended, can reach 7-8 cm. for an average sized animal; the "myones" are in a parallel arrangement and are themselves quite long.

Its chronaxie is $2-3\sigma$ (ordinary temperature, i.e. $18-20^{\circ}\text{C}$). The duration of its contraction is 1.5-2 sec., with about $\frac{1}{2}$ sec. for the ascending phase. Thus it is roughly 10 times slower than the sartorius of the frog.

I examined this muscle to see whether, like the sartorius, it had a region deprived of any nervous ramifications. For this purpose I used the traditional method; swelling in dilute acetic acid impregnation with osmic acid, mounting in glycerine. This method is sufficient, in spite of the thickness of the muscle, to follow, with a weak microscope, or better with a binocular, the distribution of intramuscular nerves, provided the muscle is pressed flat between two microscopic slides and well lighted. Unfortunately, nerve fibres are spread everywhere in the muscle. A nerveless region would have been valuable in some experiments, but for our actual problem this complex structure allows nevertheless some distinct results, as we shall see later.

This muscle, called dorso-occipital by the anatomists, has no analogy in Amphibians nor in Mammals; we need not enquire by what evolution it has been created and if it is, as very likely, the homology of all the complex series of spinal muscles; in spite of its multiple innervation, it appears in *Testudo* as an organic and functional unit; we have systematically examined its excitability with a stigmatic cathode moved over its entire length; we have found everywhere, on a given muscle, the same chronaxie.

It is reached very easily from the ventral surface; the head (with the encephalon previously destroyed through the skull) is maintained in extension as well as the four limbs; the sternal plastron is removed with a saw and cutting forceps; all the viscera thus uncovered are removed. Then the two muscles, the left and the right, appear stretched and almost parallel, separated one from another, on the head side, by the cervical vertebrae, and on the other side, converging toward the last dorsal vertebrae. When the lungs have

been removed, the muscles in question are isolated in the cavity of the dorsal carapace, accompanied only by synergic muscles, shorter, having the same dorsal insertion but attached lower on the neck. There is on each side, between this short muscle (dorso-cervical) and the long muscle (dorso-occipital) which we take as our object, a band of loose cellular tissues easily torn; nevertheless, it is better to remove these muscles both at the same time, since their separation exposes our muscle to injuries which provoke local alteration of excitability. One can, on the other hand, excite this one *in situ*, resecting the clavicles if they interfere with the manipulation of the electrodes. The nerves enter by the internal surface; their entrance constituting sometimes singular points, it is preferable to place the electrodes on the external surface. When the muscle is removed, by section of the two insertions (and also the cervical insertion of the short muscle), it is turned with the external surface upward. The muscle thus prepared is placed in a rectangular trough as we have done with the sartorius, and undergoes the same experiments.

We took as physiological solution our usual Ringer; which, maybe, is not exactly adapted to the turtle muscles but which has been found sufficiently adequate for our experiments.

Here are our results.

A. With a stigmatic electrode two curves are obtained which differ by their time constant, more than those of the *Holothuria* and less than those of the frog.

Example. Exp. of Oct. 29:

I. Stigmatic cathode: ∞ , 3.8; 100, 3.8; 40, 4.0; 10, 5.0; 5, 5.9; 2, 7.6; 1, 11.5.

II. Diffuse cathode: ∞ , 7.8-7.5; 100, 7.8-7.5; 40, 8.5-9; 20, 12; 10, 15-15.5; 6, 19.5.

A simple inspection of these figures shows the chronaxie or pseudo-chronaxie to be 2σ in the first case and 10σ in the second.

If we divide by 2 the figures obtained with the diffuse cathode and if we plot these numbers we get the curves of Fig. 4.

B. Under a block, we get, as in the case of the frog, a complex of two curves, characterized by chronaxies or pseudochronaxies of $2-3\sigma$ for one and $10-20\sigma$ for the other.

Example. Exp. of Oct. 24. 12 mm. block.

I. Block toward the middle of the muscle; anode on the cephalic side: 100, 3.2; 40, 3.7; 20, 5.2; 15, 5.6; 10, 6.6; 5, 8.0; 3, 9.5-10; 2, 11.5; 30, 4.8; 20, 5.4; 40, 3.9; 25, 3.2.

II. Block removed its width toward the cephalic end; same direction of the current:

∞ , 3.0; 100, 3.0; 40, 3.2; 20, 3.3; 10, 3.5; 5, 4.0; 3, 4.8; 2, 5.4; 1, 6.4; ∞ , 3.1; 20, 3.4.

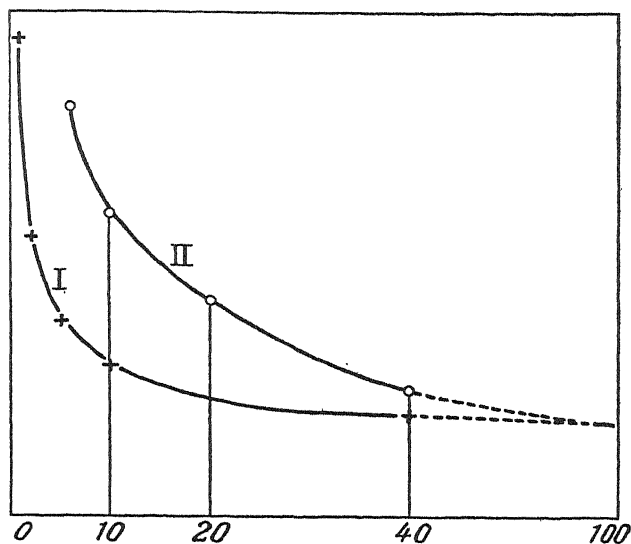


Fig. 4. Strength-duration curves from a turtle muscle: I, with stigmatic cathode; II, with diffuse cathode.

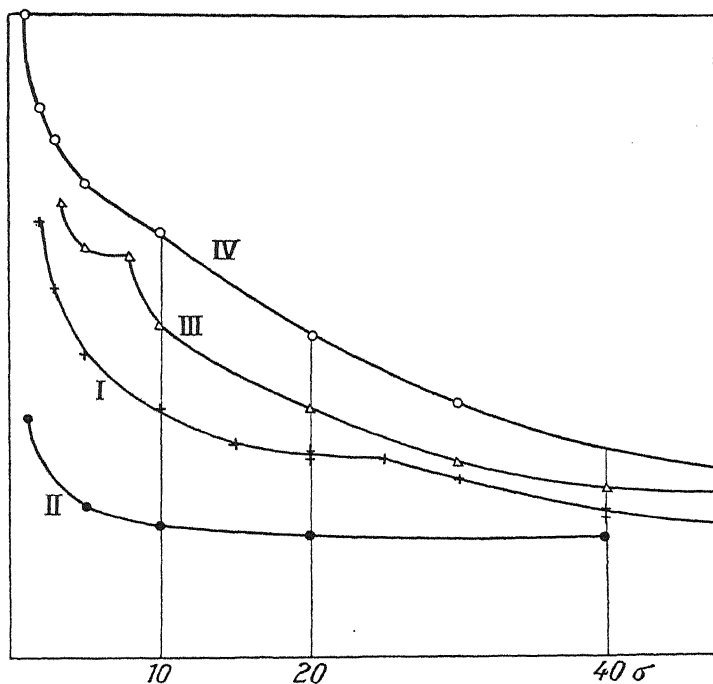


Fig. 5. Strength-duration curves from a turtle muscle under varying conditions (see text).

III. Block put back in about the same position as I; same direction of the current:

∞ , 4.0; 100, 4.0; 40, 4.4-4.5; 20, 6.6; 5, 10.8; 3, 11.5; 5, 11.0; 8, 10.6; 3, 12-12.5; 8, 11; 20, 6.3; 30, 5.0-5.3.

IV. Same position of the block; reversed current:

∞ , 4.0; 100, 4.0; 40, 5.4-5.6; 100, 4.2-4.4; 20, 8.5; 10, 11.0-11.5; 5, 12.5; 3, 13.7-14.0; 2, 14.5-15.0; 1, 17.0.

These figures plotted directly give the different curves of Fig. 5.

The chronaxies or pseudochronaxies are 1.5σ for curve II, 12σ for curve III and 10σ for curve IV; curve I is composed of two elements for which a probable extrapolation gives two time constants, one of 3σ and the other of 18σ . In short, when the turtle muscle is studied in a Ringer solution, it behaves, with regard to its strength-duration relation, as the frog muscle, giving two curves which differ from one another in their time constant. The slower one must be likened to the curve named α for the frog, not only because it is the slowest one that can be obtained, but also because it appears under the same conditions required for the appearance of α ; in the same way the more rapid curve must be likened to the γ curve of the frog because it is the most rapid obtainable and also because its determination is the same as that of γ .

The following is the important point, the significance of which we shall discuss.

The time constant of α has the same value for the turtle and for the frog, or rather oscillates according to the experimental conditions, for one as for the other, between the same limits, *i.e.* between 10 and 20σ . The time constant of γ is notably larger for the turtle than for the frog.

Let us consider now the chronaxie measurements obtained with different sizes of electrodes, using the same apparatus as above. Several measurements were often made with the same electrode placed on different parts of the muscle.

In the following table the first figure represents the inside diameter of the electrode in mm.; the second figure (and eventually the following ones when measures have been taken on several spots of the muscle), given in heavy print, represents the liminal durations for a voltage twice that of the rheobase:

Oct. 18: 1.6, **3.2**; 0.80, **2.5**; 0.40, **1.8** or **2**.

Oct. 19: 0.40, **2.2**; 0.80, **2.6**; 1.80, **3.5** or **3.9**.

Another: 2.8, **9**; 1.8, **5**; 0.30, **5**, then **4**; 1.80, **6**.

Oct. 21: 0.80, 3-2.8-2.8; 0.30, 2.5.

Same muscle later: 0.8, 2.5; 0.30, 2.2-1.5; 0.8, 2.2-2.2-2.0; 2.8, 4.5-4.5.

Oct. 25: 0.40, 1.8; 2.8, 3.2; 0.40, 1.8.

Dec. 12: 0.8, 2.3; 1.12, 2.7; 1.8, 4.5; 0.3, 2.5-2.0-2.2; 0.57, 2.5-2.5.

Dec. 13: 0.57, 2.1-2.3-2.2; 0.80, 2.6-3.0-2.8; 0.30, 2.1-2.0-2.0; 1.0, 3.2-3.7-3.2-3.5; 2.0, 4.8-4.2-4.8-4.5.

Dec. 15: 0.80, 2.7; 0.27, 2.5-2.0; 1.5, 3.0-2.8; 0.60, 2.7-2.0-3.0-2.5; 0.40, 2.1-2.0-2.2.

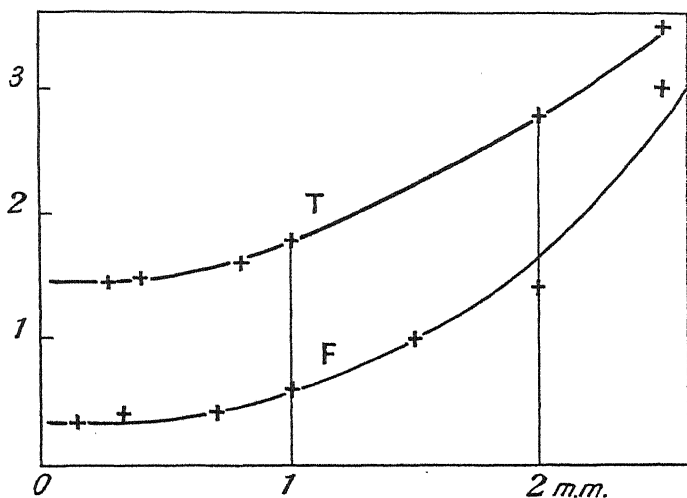


Fig. 6. Chronaxie measurements with capillary electrodes of different diameters: *F*, frog muscle; *T*, turtle muscle.

Another: 0.40, 1.5-1.5; 1.0, 1.8; 2.5, 3.5-3.8; 2.0, 2.8; 0.27, 1.4-1.5; 0.80, 1.6.

Dec. 19: muscle curarized: 0.57, 3.5; 1.0, 3.8; 2.5, 5.0.

The experiment before the last is plotted on the graph (Fig. 6). This curve is similar to the one given by the frog sartorius which we draw under *F* for sake of comparison; the ascending parts of these two curves, when the diameters of the electrodes increase, aim toward a high value of pseudochronaxie; while for the small diameters, $\frac{1}{2}$ mm. and below, each curve aims toward a limit which is much higher for the turtle than for the frog.

Other series of figures do not give as distinctly the curve previously found for the frog, but even with their irregularities they are confirma-

tive; especially whatever might be the uncertainty of the curve by which one could interpolate the experimental figures, it is always evident that when the electrodes become smaller and smaller the chronaxie or pseudo-chronaxie does not tend toward zero but toward a value around 2σ , which is the important point.

In the experiment of Dec. 12, we notice a peculiarity which I did not mention above as I believe it does not directly concern the question studied, but which is worth while to note in view of certain discussions. After the regular series of

0.8, 2.3; 1.11, 2.7; 1.8, 4.2-4.5,

the electrode 0.3 gave <1 ; but when moved a few mm. it gave 2.5. A dozen systematic trials observing carefully the disposition of the electrodes generally gave values ranging between 2.0 and 2.5. But a definite point always gave values $<1\sigma$. We noted, first, that the response at this point was very different from the others; instead of a lengthwise rise varying in amplitude with the intensity of the stimulus evidently corresponding to the contraction of a variable number of myones lying immediately under the cathode, we noticed a contraction which was large from the start in a portion of the muscle lateral to the electrode; secondly, examining the preparation with a magnifying lens, we noticed that the point which has a small chronaxie is the entrance of a nerve in the muscle, which, by mistake, had been placed with its internal surface upwards. Next we tried the electrode 0.57 which gave us, in the same way, according to the point where it was placed, a chronaxie <1 at the point formerly described or of 2.5 for any other point above or below or more on the left (the nervous point is on the right side of the muscle). The values > 2 evidently correspond to the direct normal stimulation (keeping for the word direct its usual lack of precision), but the values <1 evidently concern the nerve from the characters of the response, and nevertheless should not be considered as normal nerve chronaxies, for the condition in which they are obtained is to place the electrode just on the section of the nerve; maybe we are dealing with an injury excitability; maybe with another phenomenon which I am studying now.

GENERAL CONSIDERATIONS AND CONCLUSIONS.

The very idea of chronaxie, of a relative time varying from one physiological object to another and characterizing this object, came from the comparison of different muscles which directly exhibit different speeds of contraction. It is logical to suppose that a muscle which contracts slowly will be sensitive to a prolongation of the stimulus, while the excitability of the frog gastrocnemius, excitability which is totally displayed with a very brief stimulus, should be related to its almost instantaneous contraction. Fick was guided by this feeling when he chose the adductor muscle of the *Anodonta* to show the part played by the duration of a constant current in the stimulation process, a part hard to perceive on the quick gastrocnemius. Likewise, when Engelmann wanted to study the time function of stimulation, he chose a smooth muscle, in spite of the experimental difficulties entailed by this choice.

The relation between the speed of contraction and the speed of stimulation has been verified for the material chosen. Engelmann generalized when he observed under a microscope a series of infusoria and aquatic animalcules; including thus at the same time different forms with slow and rapid organs, he stimulated nearly at will either one or the other according to the durations of the currents used. He was referring to this experiment when he wrote the expression "physiological time," which appears to-day to be so suggestive. Unfortunately he neither developed nor explicitly interpreted this expression.

Thirty-two years later, ignoring, as everyone else, these works of Fick and Engelmann unjustly forgotten, I went over the same mental process. Having noticed that, everything else being equal, the capacity of a condenser discharge stimulating at the minimum of energy is less for the gastrocnemius of the frog than for that of the toad, the latter being less rapid, I went to Arcachon to find much slower muscles in invertebrates and prepared in advance an apparatus capable of giving much longer stimuli.

In fact, *Aplysia* required for the construction of its liminal intensity duration curve, times of hundredths and tenths of a second, its chronaxie being 100 times that of the frog gastrocnemius, for an elementary contraction (homologous to the twitch, though this usual expression for the frog cannot very well be applied here) which is also about 100 times longer.

This relation between the duration of the contraction and the duration necessary for excitation has been found every time it has been looked for. The muscles of locomotion of the turtle seemed once to be an exception; but we were dealing with a false measure of chronaxie [L. et M. Lapicque, 1927].

This is the way that chronaxie appeared in its broad aspects, that is to say the physiological significance of chronaxie in the strict sense. This conventional duration derived from the artificial phenomena of electrical excitation reveals, therefore, a profound property of the organ.

But the organ in question is complex; where we are dealing with a vertebrate muscle anatomically distinct or with a part arbitrarily cut in the foot or the mantle of a mollusc, when the electrode is directly applied on the muscle, the current is bound to reach both elements. And then the centenarian problem of the distinction between the nerve excitability and the muscle excitability is presented in a new form. The chronaxie which we have noted to be in accord with the duration of the contraction, is it the chronaxie of the muscle or that of the nerve?

The agreement between the muscular chronaxie and the contractility was self-evident, but if it is a question of the chronaxie of the nerve, we are dealing with a most remarkable fact: the accord between the excitability of one substance and the functional characteristic of another. The existence of this accord as a rule cannot be contested for voluntary nerves and muscles, whatever the interpretation given to the so-called direct stimulation.

With the simplest and most ordinary apparatus, such as metallic wire as cathode, we get the same chronaxie when the active electrode is applied on the muscle or on the nerve.

Consequently, the chronaxie given by the so-called direct stimulation should be either the chronaxie of the nerve or that of the muscle equal to the chronaxie of the nerve; in either case there is an accord between the excitability of the nerve and contractility of the muscle.

This point of view does not seem to have been expressed before, though it could have been thought of 25 years ago in relation to a remarkable series of experiments entirely independent of the present discussion, published just at the time when the idea of chronaxie was being developed by Keith Lucas and ourselves. When measuring the speed of the impulse in a series of motor nerves and at the same time the duration of the corresponding muscle twitch, Carlson [1906] noticed that these two values are inversely related; a quick muscle is innervated by a quick nerve, a slow muscle by a slow nerve.

Does not this chronological accord between these two functions imply the accord between the two excitabilities?

In fact the relation between the speed of the nervous impulse and the nerve chronaxie, deducted first from Carlson's experiments, has been largely verified. The relation between the muscle chronaxie and the duration of the contraction, admitted as evident ever since Fick, has never been contested in principle; but if the nerve-muscle isochronism is not accepted, we might say that this relation has received no experimental demonstration, all the experiments considered as demonstrating it being nothing but the comparison between the duration of the contraction of the muscle and the chronaxie of the nerve.

It seemed to me that we had here a means of testing the significance of the α curve. Studied only on rapid muscles (the difference between the toad and the frog is negligible when compared with the variability of a single muscle), it has been interpreted as indicating a notably larger muscle chronaxie than nerve chronaxie. But relation between excitabilities does not necessarily mean equal chronaxies. If the

α curve represents the time function in the excitability of muscle (and even according to Rushton [1932 *d*], it would be the only means to account for it), its time constant, whether it is called chronaxie or not, must appear longer for the slow muscle than for the rapid one, increased proportionally to the nerve chronaxie, or, at least, exhibiting in each case an excess of the same order of magnitude above the nerve chronaxie.

Such a research, attempted in the present paper, has given a strikingly clear answer: the time constant of the α curve is absolutely independent of the duration of the muscle contraction, while chronaxie, measured on the same muscle with my ordinary technique, increases regularly with this duration. Thus the chronaxie goes from about 0.3σ for the frog muscle to $2-3\sigma$ for the muscle of the turtle which has a contraction 10 times slower; then to 9σ for the *Holothuria* muscle which is 40 times slower. That is to say, considering my chronaxie, we find on these muscles the fundamental relation on which I insisted at the beginning of this part of the present article.

On the contrary, the time constant α remains the same for the turtle and for the *Holothuria* as well as for the frog, varying according to undeterminable conditions, between 10 and 20σ with no systematic difference, when the durations of the contractions varied from 0.10 or 0.20 sec. for the frog, to 1.5 or 2 sec. for the turtle and to 6 or 8 sec. for the *Holothuria*.

I do not see how one could refuse to homologate these curves of the slow muscles with the α curves of the rapid muscles. Not only are the conditions of production the same—arrival of the electric current lengthwise through an electrolytic bath which constitutes a very large fluid electrode, with or without Rushton's block; but also the appearance of the curves, their irregularities as well as their general shape are the same.

In any case, we do not obtain a single curve more elongated on the time axis. So that whether the muscle is 10 times, 40 times slower or quicker, it always gives the same α curve.

This shows how right I was when I refused to consider as a chronaxie the time constant of α obtained with a large fluid electrode. It is no longer a question of a criterion drawn from the shape of the curve, not even of an alteration in the measurement due to the geometrical or physical conditions of the experiment. It is a question of the essential signification of the term chronaxie, *i.e.* relative value of time, a measure of the "physiological time" of Engelmann; α is not only a wrong measurement of this time, but it is in no way a function of it.

What I said about chronaxies is valid for the γ curves; they occupy

on the scale of time a situation in regard to the slowness or quickness of the muscle on which they were measured; γ for the turtle is placed between the γ of the frog and the α common to all muscles. For the *Holothuria* γ is very close to α , so close that at times it cannot be distinguished from it, the chronaxie of this muscle being very close or equal to time constant of α^1 and the individual cases where γ is farther apart from α are precisely the cases when the chronaxie is distinctly smaller than the constant α .

In short, what has been called the γ excitability or γ substance is the thing which is characterized by the chronaxie.

If one wanted to continue to identify the γ substance with the nerve and the α substance with the muscle, one would sink in a strange paradox: accord between the nerve excitability and the muscle contractility with no part given to the muscle excitability in this accord.

Moreover, a γ curve and correlatively, a chronaxie equal to that of nerve, can be given by the muscular substance alone as is the case for the pelvic end of the frog sartorius. But, for this measure, it is necessary to use a stigmatic cathode in the air or in the Ringer (*i.e.* of an effective diameter, not over $\frac{1}{2}$ mm. [Lapicque, 1932, p. 265]).

Jinnaka and Azuma [1922] were the first to show such a small chronaxie on this tissue with a pore electrode of 10 σ diameter. Rushton does not deny this fact; on the contrary, he seems to be astonished that I took the trouble to verify the results of these Japanese authors which he considers as accepted. But he sees here, from his point of view, not a true γ curve but a shortened α curve. The argument is not purely verbal; taking exactly the counterpart of my reasoning, it appears as equivalent and so might leave the choice undetermined.

I say: a large electrode introduces a parasitic physical phenomenon (retrograde polarization) which increases the time necessary for the current to achieve the process of stimulation; thus appears a false

¹ I must confess that I was surprised by this last result, having admitted that a certain physical phenomenon which I called retrograde polarization [Lapicque, 1932] is introduced between the moment when the electric current is applied and the actual stimulation. I thought that the sum of these two processes would always give longer durations than the actual stimulation time, in other words that the constant of α would always be longer than that of γ . To tell the truth, I had added that we were not dealing with a pure and simple addition of the necessary times for each process [Lapicque, L. et M., 1932], and, on the other hand, that their complexity did not allow us to foretell theoretically the resultant of their conflict [Lapicque, L., 1932, p. 274]. But if the theory of the α stimulation has become more obscure with the experiments on slow muscles, its physiological interest has also faded.

chronaxie which is too large. Only a small electrode can give the true muscle chronaxie.

Rushton [1932 *c*] says: a small electrode introduces a parasitic physical phenomenon (lateral diffusion of polarization) which limits the time during which the current may increase the polarization causing the stimulation; thus appears a false chronaxie, which is too small. Only a large electrode can give the true muscle chronaxie.

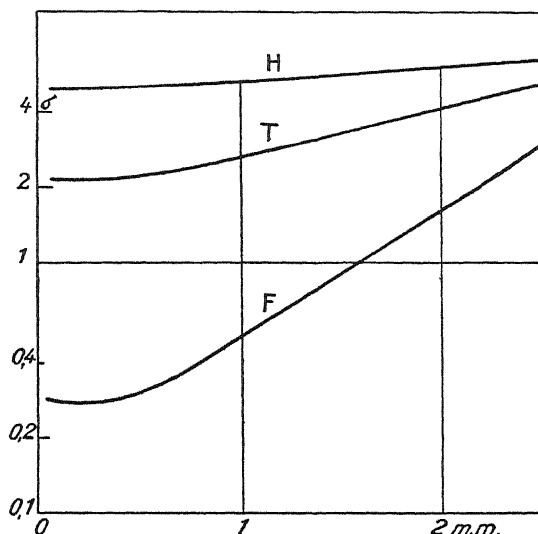


Fig. 7. Schematic graph showing the influence of the size of the electrode: *F*, on a frog muscle; *T*, on a turtle muscle; *H*, on a *Holothuria* muscle.

I had in advance [Lapicque, 1932, paper published after the one of Rushton cited above, but the proofs had been corrected already beforehand] objected to any such conception an experimental fact: that on a rapid muscle when the diameter of the electrode is indefinitely reduced, the chronaxie or pseudochronaxie does not decrease indefinitely, but remains constant below a certain diameter, still of considerable size. After the publication of Rushton's paper, I thought of opposing this theory of lateral diffusion by a quantitative discussion, for it is very unlikely that such a phenomenon might intervene appreciably in times as short as the thousandth of a second for distances of the order of the millimetre. But this theoretical discussion becomes useless now that the comparison between slow and rapid muscles, which is the true way of studying the chronaxie, gives us a peremptory argument. With the same

series of decreasing size electrodes, the smallest chronaxie we get for the smallest electrode is, instead of 0.3σ for the frog muscle, $2-3\sigma$ for the muscle of the turtle, $5-6\sigma$ for the muscle of the *Holothuria* (these last measures have been made on most rapid specimens). Starting from here toward larger electrodes, the liminal duration for a voltage twice that of the rheobase increases much more slowly for the turtle than for the frog, while it increases very little or not at all for the *Holothuria*.

These facts are represented in a somewhat schematic way, but very close to the experimental figures, in Fig. 7. The abscissa represents the diameter of the electrodes, the ordinate represents the chronaxie or pseudochronaxie in logarithms (the relations being in this case more significant than the differences). If the measure of time characteristic for each muscle was given by the large electrodes and this measure falsified in the case of small electrodes according to Rushton's conception, the curves would reach very different heights on the side of large electrodes, they would converge where the size of electrodes tends toward zero. But we see exactly the contrary; on the side of small electrodes they reach different heights which correspond to the relative speed of the muscles and converge on the side of large electrodes; the common value towards which they aim is evidently nothing but α , which, as we have seen, has the same value for all the muscles.

To maintain that the true muscle chronaxie is only given by large electrodes would make it necessary to admit the same chronaxie for slow and for rapid muscles; which would mean the abandonment of any conception of chronaxie.

Parenthesis on curare.

In our general consideration of the relations between contractility and excitability we cannot avoid mentioning the case of curare. Everyone admits that the contractility of a curarized muscle is not changed. Is its excitability changed? The formerly classical theory said no, precisely on account of the absence of change in the contraction; but a whole series of experimental facts proves that the muscular excitability is changed; the chronaxie is increased; these facts have been contested, but the above discussion results in validating them. There seems to be therefore, in the muscle itself, a break in the relation between excitability and contractility. There is no difficulty in accounting for this, as several recent authors have done by localizing separately contractility and excitability, the first evidently in the anisotropic substance, and the second in the sarcoplasm.

The classical authors of the past generation at times gave their opinion in the following way: the contractile substance is left untouched by curare; I have nothing to say against this formula; the inaccuracy starts when the contractile substance is implicitly assumed as being the totality of the muscle.

One might regret that curare introduces such a complication in our question. But if this complication did not exist, that is to say if curare would slow down the contractility at the same time as it does the excitability, its way of action would have been very clear even at the time of Claude Bernard. It would have been useless to call upon the mythical motor end-plates; and perhaps Brücke, whose attention had so deeply seized the part played by time in the excitation process, might have seen the normal isochronism and its alteration, instead of declaring an essential heterochronism which is unveiled by curare. No matter what it be, as this complication appears as a fact, we ought to explain it; the localization of the excitability in the sarcoplasm opens the way to explanation, raising besides some interesting considerations in themselves. But this paper is not the place to develop such a theory.

Incidentally I note a consequence of the present work which is related to the discussions on curare; as the α curve does not change from one muscle with a small chronaxie to another muscle with a large chronaxie, the fact that the α curve remains the same after curarization cannot be called upon to demonstrate that the muscular excitability has remained unchanged.

SUMMARY.

Two slow muscles with parallel fibres have been studied in the same conditions which, in the case of the frog sartorius, produce intensity-duration curves called α and γ .

On the retractor of the head of the turtle (duration of the contraction 1.5–2 sec., chronaxie measured in the air 2–3 σ) the α curves have a time constant which varies between 10 and 20 σ ; the γ curves correspond to the chronaxie.

On the longitudinal muscle of the *Holothuria* (duration of contraction, 6–8 sec., chronaxie, 5–10 σ), the α curves have a time constant which varies between 10 and 20 σ ; the γ curves are hardly distinct from them.

As these muscles give α curves not at all different from the α curves of the frog muscle, though they are respectively 10 and 40 times slower, we conclude that the α curve has nothing to do with the chronological properties of the muscle and is in no way a chronaxie.

Incidentally it was shown that a group of facts, which every one admits, demonstrates that the excitability of the motor nerve is chronologically in accordance with the contractility of the corresponding muscle. It would be strange if the muscle excitability took no part in this relationship.

We have studied, on these same muscles, the influence of the size of the electrode upon the liminal duration for a voltage twice that of the rheobase; this influence becomes less and less appreciable the slower the muscle; the large electrodes give values which are relatively slightly different whatever the muscle considered; the gradual decrease of the diameter of the electrodes gives values tending for each muscle toward an inferior limit which is higher the slower the muscle. Thus we conclude that the muscle excitability can be chronologically characterized only with small electrodes.

AGAIN A POST-SCRIPTUM VERSUS RUSHTON.

As I was correcting the proof of this article there appeared another article by W. A. H. Rushton entitled "Lapicque's theory of curarization" (this *Journal*, 1933, 77, 337). According to this author, this theory is untenable, all arguments in its favour are incorrect, and even the experimental results which support it are invalid. But this criticism is as unjustifiable fundamentally as it is striking in form. All of the objections of Rushton can be shown to be false, and each of his experiments and so-called controls are open to serious criticism which deprives them of significance. Such a discussion would be too long to be given here. But I wish to show at once, by an example, the value of the logic and documentation represented in this article of Rushton.

In order to show that the large chronaxie obtained on the muscle after the action of curare did not exist before the action of the poison, I made use of progressive currents according to the following law: The smaller the chronaxie of a tissue, the greater is the decrease in effectiveness of electrical stimulation due to a retardation in the establishment of the current. Rushton comments that "this is a very bold assumption and some substantiation would not have been out of place."

It is true that in my article of 1925, cited by Rushton, a short and preliminary note, I did not substantiate this law supposedly already accepted, but in my book of 1926, a book often quoted by Rushton, immediately following the expression of the above law is added "j'ai exposé cette loi avec divers exemples dans le chapitre VII." Here Rushton would have found eight pages devoted to the substantiation he asks for. It is difficult to understand how he could have written such a sentence as "Whether Lapicque's generalization has in fact ever been supported by a series of investigations or not, I do not know."

For this chapter VII cites first an experiment of Fick [1860] (since it is necessary to go back to this old classic to find the origin of "this bold assumption"); then an experiment of von Kries [1884], then, with reproduction of figures, two experiments of Keith Lucas [1907], which show clearly the law by comparison of the frog and the toad, also a series of experiments which I have carried out systematically on this subject, and further, in the chapter where I made the application under discussion, are cited Cardot and Laugier [1913].

But Rushton, being not aware of any of these duly cited investigations, discovered another one: "The only experiments I know which test the matter directly [Lucas, 1908] show quite contrary results." These experiments of Lucas, cited unequivocally in the final references of Rushton, are not those cited in my book, but they give exactly the same results, in the sense that I have indicated and do not show any contrary results as Rushton has imagined by an inconceivable aberration.

Would not the discussion one by one of such objections be tedious for the reader? And, on the other hand, it seemed useless to present the author himself with arguments, for he gives them little or no consideration. I was, at first, planning not to misuse, with a detailed answer, the hospitality already generously offered by this *Journal*, but I heard that some physiologists of note have been impressed by one or another of Rushton's arguments. Consequently, every one of these arguments must be formally criticized. This shall be the subject of a following article.

In the meantime I will cite a few points as a matter of fact.

The above article presents new experimental arguments which many physiologists have judged to be conclusive. They may be applied to the theory of curarization, as I have noted in the parenthesis on curare, which was in the hands of the editors before I knew of Rushton's attack on this point. For instance, it is now quite clear that the time constant α should not be increased by curarization.

But, for seventy years, all physiologists stimulating with dry electrodes have found a slower excitability after curare, and Boehm like the others, contrary to Rushton's assertion. Recently, being anxious to test the phenomenon also in Ringer, I found unequivocal confirmatory results.

Moreover, taking up again my experiments on antagonism between veratrine and strychnine, I obtained once more the results which have been denied, and besides, demonstrative graphs, under some given conditions, which are in accord with the theory. And I will give in advance the conclusion I intend to vindicate; I do not find in Rushton's paper anything which actually disturbs the basis of the chronological theory of curarization.

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THE INFLUENCE OF AMMONIUM CHLORIDE ON ADAPTATION TO LOW BAROMETRIC PRESSURES.

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DURING the recent successful ascent of Mount Kamet (25,447 ft.) Greene [1932] found that he seemed to obtain some benefit at the high altitude in his own case by the daily administration of small doses of ammonium chloride (7 grains or 0.45 gram three times daily), and he was the only member of the climbing party who did not lose weight. Another member of the party who was clearly affected by the altitude showed a rapid improvement in condition when he was treated with ammonium chloride in the same way. That treatment with ammonium chloride might prove beneficial during acclimatization to a high altitude had been suggested by J. S. Haldane [1922] for the following reason. The low partial pressure of oxygen in the air breathed causes hyperpnœa which in its turn induces an alkalosis, but the kidney by excreting a less acid urine than normal tends to restore the reaction of the blood to its normal value. The more perfect this compensation the greater will be the hyperpnœa due to the deficiency of oxygen since alkalosis results in a diminution of the activity of the respiratory centre, and the greater the hyperpnœa the lower will be the alveolar CO_2 pressure and—a matter of very great importance at the low barometric pressures encountered at high altitudes—the higher the alveolar oxygen pressure. A final equilibrium will be attained when a balance is struck between the two opposing influences; the kidneys will nearly compensate for the alkalosis caused by the hyperpnœa due to deficiency of oxygen, the hydrogen ion concentration of the blood will be but a trifle below the normal value, but the hyperpnœa during rest will be considerably greater than it was at first. The slow adaptation of the breathing can be well seen in the results obtained by Douglas, Haldane, Henderson and Schneider [1913] during the Pike's Peak expedition: there is a sharp drop in the alveolar CO_2 pressure which is immediately evident on reaching the

altitude of 14,000 ft., and this is succeeded by a secondary further drop which is not completed till a number of days have elapsed, the reduction of alveolar CO₂ pressure being accompanied by a proportional increase in alveolar oxygen pressure. Time is required for the kidney to bring about the final accommodation, and during this time it is virtually restricting the acid excretion from the body. The addition of acid to the body might therefore be expected to hasten this accommodation. As J. B. S. Haldane [1921] has shown the ingestion of ammonium chloride is one of the most effective ways of bringing about an acidosis.

In view of the forthcoming attack on Mount Everest we decided to see whether the conclusions tentatively reached by Greene on Mount Kamet could be supported by more direct experimental evidence, and for this purpose we have made experiments on the influence of reduced barometric pressure on a subject who was in some cases given ammonium chloride beforehand.

Our experiments were made in the steel chamber in the Sir William Dunn School of Pathology at Oxford, and we take this opportunity of thanking Prof. Dreyer for putting it freely at our disposal. This chamber was large enough to contain two persons in comfort as well as a Martin bicycle ergometer and other apparatus, while large windows afforded an excellent view of the interior from the outside. We are indebted to Mr Jesse Wheal for running the exhaust pumps and for the care which he took to maintain the barometric pressure within the chamber at the desired level.

Kergin acted as the subject of experiment throughout. He was 25 years of age, weight 160 lb., of athletic build, and though accustomed to take a certain amount of active exercise was in no sense in muscular training. He was accompanied in the chamber by Greene who began to breathe through a closed oxygen circuit fitted with a large vessel of caustic soda for the absorption of CO₂ as soon as the barometer had fallen to about 508 mm., and continued to breathe through this circuit until the pressure was raised again at the end of the experiment. This not only gave us an observer inside the chamber who was not exposed to deficiency of oxygen but allowed those outside a good standard with which to compare the degree of cyanosis developed by Kergin.

After four preliminary experiments, in the first and third of which 5 g. of ammonium chloride had been taken beforehand without any striking result, we adopted the following procedure. After the subject had sat still for a short time samples of his normal alveolar air were taken. He and Greene then entered the chamber and the pressure was

at once reduced. The reduction of pressure was made in successive stages to barometric pressures of 506, 421, 375 and finally 347 mm. (equivalent to an altitude of 20,000–22,000 ft.). A pause of about 10 min. was made at the barometric pressure of 506 mm., one of 15 min. at the barometric pressure of 421 mm., and one of about 9 min. at the barometric pressure of 375 mm., in order to allow time for the hyperpnœa resulting from the diminution of oxygen pressure to reduce the general CO_2 level in the body in correspondence with the diminished alveolar CO_2 pressure. After reaching the lowest barometric pressure a period varying from $11\frac{1}{2}$ to $20\frac{1}{2}$ min. was allowed to elapse and then alveolar samples were collected from the subject whilst he sat at rest. Immediately after this he mounted the bicycle ergometer and began to work in time to a metronome at the rate of 5000 ft. lb. (690 kg.m.) per minute. After completing the work he again sat down and some 10 min. later alveolar samples were again taken. The pressure in the chamber was then raised to normal atmospheric pressure and further alveolar samples were taken about 25 min., and in some cases 50 min. in addition, after leaving the chamber. Pulse and respiration rates were noted before and after doing the muscular work in the reduced pressure.

The figures given for the composition of the alveolar air represent the average of alveolar samples given one at the end of a normal inspiration and the other at the end of a normal expiration as in the usual Haldane-Priestley procedure. In the first experiment we took only single pairs of such samples, but in order to obtain greater accuracy in subsequent experiments the preliminary determinations before entering the chamber were made in duplicate in the second experiment and in triplicate thereafter, the determinations in the reduced pressure before the work as well as those taken after returning to normal atmospheric pressure were made in duplicate (save for the determination made after leaving the chamber in the second experiment which gives the result of but a single pair of samples), and in Table I the figures represent the average of such duplicate or triplicate determinations. The values given for the composition of the alveolar air 10 min. after the work was finished rest on single pairs of samples: these samples were taken merely to give a rough idea as to the influence of the muscular work on the alveolar air.

As regards the dose of ammonium chloride we were faced with a difficulty. With a large dose there is no doubt that we should have obtained a very obvious result, but ammonium chloride is a gastric irritant and a dose which might perhaps be just tolerable at sea level

would be likely to become impossible when associated with the anorexia encountered at really high altitudes. If therefore our results were to be of any practical importance in respect to the acclimatization of the mountaineer we were bound to restrict the dose. At the same time we could certainly exceed the dose that might in practice be advocated for this purpose. When ammonium chloride is administered to a normal subject at sea level the kidneys by excreting excess of acid tend to oppose the efforts to create an acidosis; at a high altitude on the other hand when the kidneys may be, as pointed out above, virtually retaining acid in the body, introduction of acid into the body should be an assistance to the kidneys. The observations of J. B. S. Haldane [1921] show that the acidosis caused by the ingestion of ammonium chloride takes time to develop and that it may remain perceptible for many hours. We therefore decided to give the ammonium chloride in three doses, 5 g. at lunch time and another 5 g. at dinner time on the day preceding the experiment and a further 5 g. at breakfast time 2 hours before the beginning of the experiment. The ammonium chloride was taken directly after the meals in fairly strong solution followed by a drink of water, in this way avoiding the gastric irritation which may be caused if it is taken on an empty stomach.

The general course of the experiments, the barometric pressure in mm. mercury and the results of the alveolar air analyses are shown in Table I. It will be seen that the initial resting alveolar air samples taken at normal barometric pressure in the experiments in which no ammonium chloride was taken show a definite variation. Whereas in the experiments made in November and December the alveolar CO_2 pressure was about 38.5 mm. low values of 35.5 and 36.8 mm. were shown in January. Another control experiment on December 20 had to be hurriedly terminated, as the subject, who had complained of feeling cold while the pressure was being reduced in the chamber, developed a definite rigor when he stopped the muscular work. The pressure was at once raised to normal and within an hour of leaving the chamber his temperature had risen to 102°F . The cause of this was influenza. The attack was a mild one and the subject recovered in a day or two. Whether or not the low normal alveolar CO_2 pressures recorded in January are in any way dependent on this attack of influenza we do not know. All the experiments were made at about the same time, viz. 2 to 3 hours after a meal, and any effect attributable to the secretion of the digestive juices may have varied in different experiments, but from the point of view of the practical application of our results we felt it undesirable

TABLE I.

Exp. 1, Nov. 26.		Exp. 2, Nov. 30.		Exp. 3, Dec. 8.	
Before start		5 g. NH_4Cl 2 p.m., Nov. 29. 5 g. NH_4Cl 7.50 p.m., Nov. 29. 5 g. NH_4Cl 9 a.m., Nov. 30.		763 mm. Alv. CO_2 = 5.44 p.c. = 38.4 mm. Pressure reduced	
Zero	Zero	753 mm.	Before start	Zero	763 mm.
6 min.	4½ min.	508 mm.	Alv. CO_2 = 4.87 p.c. = 34.4 mm.	4 min.	506 mm.
12½ "	15 "	Pressure reduced	Pressure reduced	"	Pressure reduced
15 "	17 "	Pressure reduced	Pressure reduced	14 "	Pressure reduced
20 "	32 "	Pressure reduced	Pressure reduced	16 "	Pressure reduced
31½ "	32½ "	374 mm.	375 mm.	31½ "	375 mm.
40 "	42 "	Pressure reduced	Pressure reduced	32½ "	Pressure reduced
41½ "	43 "	346 mm.	347 mm.	41½ "	Pressure reduced
53 "	55½ "	Alv. CO_2 = 8.66 p.c. = 25.9 mm.	Alv. CO_2 = 8.36 p.c. = 25.1 mm.	42 "	347 mm.
55 "	59 "	Alv. O_2 = 10.71 p.c. = 32.0 mm.	Alv. O_2 = 11.21 p.c. = 33.6 mm.	42½ "	Alv. CO_2 = 9.08 p.c. = 29.0 mm.
62 "	69 "	Work stops	Work starts	43 "	Alv. O_2 = 9.61 p.c. = 28.8 mm.
63 "	69½ "	Work stops	Work stops	56½ "	Work starts
70 "	78½ "	Work restarts	Alv. CO_2 = 7.41 p.c. = 22.2 mm.	60½ "	Alv. CO_2 = 8.05 p.c. = 24.2 mm.
72 "	80½ "	Pressure raised	Alv. O_2 = 11.89 p.c. = 35.7 mm.	85 "	Alv. O_2 = 10.14 p.c. = 30.4 mm.
77 "	91 "	752 mm.	Pressure raised	87 "	Pressure raised
87 "	91½ "	Alv. CO_2 = 5.08 p.c. = 35.8 mm.	753 mm.	99½ "	762 mm.
108 "	110 "		Alv. CO_2 = 4.64 p.c. = 32.8 mm.	124 "	Alv. CO_2 = 5.38 p.c. = 38.4 mm.
Exp. 4, Dec. 16.		Exp. 5, Jan. 13.		Exp. 6, Jan. 24.	
Before start		5 g. NH_4Cl 1.45 p.m., Dec. 15. 5 g. NH_4Cl 7.45 p.m., Dec. 15. 5 g. NH_4Cl 9.15 a.m., Dec. 16.		773 mm. Alv. CO_2 = 5.07 p.c. = 36.8 mm.	
Zero	Zero	765 mm.	Before start	Zero	773 mm.
5 min.	4½ min.	506 mm.	Alv. CO_2 = 4.96 p.c. = 35.5 mm.	4½ min.	506 mm.
15 "	14½ "	Pressure reduced	Pressure reduced	14½ "	Pressure reduced
16 "	16½ "	Pressure reduced	Pressure reduced	16½ "	Pressure reduced
31 "	31 "	Pressure reduced	Pressure reduced	31½ "	Pressure reduced
32½ "	32½ "	375 mm.	375 mm.	32½ "	375 mm.
41 "	41½ "	Pressure reduced	Pressure reduced	41½ "	Pressure reduced
42 "	43 "	347 mm.	347 mm.	42½ "	347 mm.
60½ "	61½ "	Alv. CO_2 = 7.86 p.c. = 23.6 mm.	Alv. CO_2 = 8.63 p.c. = 25.9 mm.	63 "	Alv. CO_2 = 8.93 p.c. = 26.8 mm.
63 "	64½ "	Alv. O_2 = 12.05 p.c. = 36.2 mm.	Alv. O_2 = 10.88 p.c. = 32.6 mm.	63½ "	Alv. O_2 = 11.03 p.c. = 33.1 mm.
80½ "	80½ "	Work stops	Work starts	66½ "	Work stops
82½ "	82½ "	Alv. CO_2 = 7.32 p.c. = 22.0 mm.	Alv. CO_2 = 7.97 p.c. = 23.9 mm.	76½ "	Work stops
95 "	94 "	Alv. O_2 = 11.61 p.c. = 34.8 mm.	Alv. O_2 = 10.58 p.c. = 31.7 mm.	78½ "	Alv. CO_2 = 8.25 p.c. = 24.8 mm.
103 "	103 "	Pressure raised	Pressure raised	90 "	Alv. O_2 = 10.64 p.c. = 31.9 mm.
108 "	103 "	765 mm.	763 mm.	98 "	Pressure raised
128 "	130 "	Alv. CO_2 = 4.16 p.c. = 29.9 mm.	Alv. CO_2 = 4.73 p.c. = 33.8 mm.	125 "	773 mm.
153½ "	155 "	Alv. CO_2 = 4.21 p.c. = 30.2 mm.	Alv. CO_2 = 4.50 p.c. = 32.2 mm.	150½ "	Alv. CO_2 = 4.73 p.c. = 34.3 mm.

that the subject should be in the post-absorptive state. In order to reduce the possibility that the subject might become naturally acclimatized to the low barometric pressure a number of days were allowed to intervene between the different experiments.

The lowest initial alveolar CO₂ pressures at normal barometric pressure are shown in the two experiments in which ammonium chloride was taken. The average of these is 34.0 mm. as compared with 37.4, the average of the four experiments in which no ammonium chloride was taken. The evidence of an ammonium chloride acidosis is therefore definite though the degree of acidosis is not very great.

Turning to the alveolar air determinations made at the barometric pressure of 347 mm. before doing muscular work it will be noted that in Exps. 1, 2 and 3 the samples were collected 11½–14½ min. after attaining this pressure, while in Exps. 4, 5 and 6 an interval of 18½–20½ min. was allowed to elapse. The extent to which the alveolar CO₂ pressure will fall in short experiments of this type will very likely be influenced by the actual duration of exposure, and this possibility must be taken into account. The average of Exps. 1 and 3 gives an alveolar CO₂ pressure of 27.5 mm. and an oxygen pressure of 30.4 mm.; in Exp. 2 in which ammonium chloride had been taken the corresponding figures are CO₂ pressure 25.1 mm. and oxygen pressure 33.6 mm. Exps. 5 and 6 show an average alveolar CO₂ pressure of 26.4 mm. and oxygen pressure of 32.9 mm., while Exp. 4 in which ammonium chloride had been taken shows CO₂ pressure 23.6 mm. and oxygen pressure 36.2 mm. Taking average values for the whole of the experiments the analyses of the resting alveolar air show in the case of the experiments in which no ammonium chloride was taken—CO₂ = 8.98 p.c. or 26.9 mm. and O₂ = 10.56 p.c. or 31.6 mm., and in the case of the two experiments in which ammonium chloride was taken beforehand—CO₂ = 8.11 p.c. or 24.4 mm. and O₂ = 11.63 p.c. or 34.9 mm. In the experiments without ammonium chloride the reduction of the resting alveolar CO₂ pressure below the value shown at normal barometric pressure is typical of the influence of lowered barometric pressure. In the experiments made after ingesting ammonium chloride the further reduction of the resting alveolar CO₂ pressure by about 2.5 mm. shows that the addition of acid to the body has resulted in an increase in the pulmonary ventilation, and in consequence of this the alveolar oxygen pressure is about 3.3 mm. higher than in the experiments without ammonium chloride.

A gain of 3.3 mm. in the alveolar oxygen pressure may at first sight appear but trivial. That would be true at normal barometric pressure,

but the very low concentration of oxygen in the alveolar air when the barometric pressure is 347 mm. must be taken into account. Examination of a dissociation curve of normal human blood *in vitro* will show that with an oxygen pressure of 31.6 mm. the hæmoglobin would not be much more than half saturated with oxygen and that an increase of 3.3 mm. in the oxygen pressure might increase the oxygen saturation of the hæmoglobin by as much as 7 p.c. At very high altitudes a very small increase in the alveolar oxygen pressure may therefore be of the greatest significance.

Judging from the data published by Miss FitzGerald [1913, 1914] about the composition of the alveolar air of residents at altitudes varying from sea level to 14,000 ft., we might expect to find an alveolar CO_2 pressure of 22 mm. and an alveolar oxygen pressure of 36 mm. after full acclimatization to a barometric pressure of 347 mm. There is still not enough information available to show whether it is permissible to extrapolate from Miss FitzGerald's data which show that up to 14,000 ft. the reduction of alveolar CO_2 pressure bears a linear relation to the fall of barometric pressure, and it is possible that at very low barometric pressures the alveolar CO_2 pressure may fall at an increasing rate, but we can at least claim that the ingestion of ammonium chloride has resulted in the pulmonary ventilation being brought measurably nearer to what it would be after full acclimatization.

The alveolar air analyses made 9-12 min. after stopping the work show a further fall of CO_2 pressure and rise of oxygen pressure. But the difference between the two series of experiments is still maintained, for the two ammonium chloride experiments show an alveolar CO_2 pressure which is on the average 2.2 mm. lower and an alveolar oxygen pressure 4 mm. higher than in Exps. 3, 5 and 6 in which no ammonium chloride was taken.

The alveolar air analyses made some 25 min. after reaching normal atmospheric pressure show that though the alveolar CO_2 pressure has risen considerably above the low values shown at the reduced barometric pressure it is still a mm. or two below the original value obtained at the start of the experiment, and this difference is still evident 50 min. after reaching normal barometric pressure. As a rule normal alveolar CO_2 pressure is quickly regained after short exposures to want of oxygen, although when full acclimatization to a high altitude has been established the alveolar CO_2 pressure returns but slowly to its normal level on descending to sea level. In the experiments described here the slow recovery of the alveolar CO_2 pressure may be, in part at least, an after-

effect of the muscular work. In any case the results in the ammonium chloride experiments point to a persistence of the acidosis due to this salt.

In the experiments without ammonium chloride cyanosis was just perceptible in the resting subject at a barometric pressure of 506 mm., distinct at 421 mm. and deep at 347 mm. before beginning the muscular work. In the two experiments with ammonium chloride the degree of cyanosis was definitely less, a fact which was especially obvious at the lowest barometric pressure. Observers inside and outside the chamber were in agreement about this. As the analyses were not done until the whole experiment had ended we were not prejudiced by a knowledge of the alveolar oxygen pressure, and in the first ammonium chloride experiment we had no idea whether or not we had given a large enough dose to produce a material effect. The subject's face was slightly flushed when sitting at rest which helped us to appreciate the depth of the cyanosis, but as soon as he started to do muscular work he became much paler and the cyanosis became more of the lilac type associated with severe anoxæmia than the full blue deep cyanosis exhibited at rest. This change in colour made it difficult to estimate differences in the degree of cyanosis, and though one observer thought the colour was slightly better in Exp. 2 than in Exps. 1 and 3 it would be fair on the whole to say that during the work the ammonium chloride made no appreciable difference to the cyanosis, which if anything became a little more pronounced as the work was prolonged. Yet it was noticeable that whilst the subject was doing work he looked far more cheerful and confident and seemed to pay more ready attention to his surroundings in the ammonium chloride experiments than in those experiments in which no ammonium chloride had been taken: indeed in the latter experiments he always looked rather anxious and gave the impression to the observers that he had to keep his attention directed to the work and had less interest in what was going on around him.

The work was maintained at the same rate, viz. 5000 ft. lb. per minute, with 62 revolutions of the pedals per minute, in each of the experiments. There was always a little difficulty in picking up the rate of the metronome during the first minute, possibly because it was troublesome to adjust the breathing to the rate of pedalling as panting developed, but after this the subject kept excellent time for a considerable period. The points noticed in the different experiments were as follows.

Exp. 1. Told to stop after 7 min. Rather tired. After 2 min. rest did another 2 min. work. A little shaky on dismounting.

Exp. 2 (ammonium chloride). In good condition throughout.

Surprised when told to stop after $10\frac{3}{4}$ min., as he felt he could have continued. Steady on dismounting.

Exp. 3. In good condition for first few minutes. Legs getting tired after 8 min. and work now seemed to involve more effort as there was a good deal of body movement which made the ergometer rock. From the tenth minute onwards obviously becoming more shaky and getting behindhand with the metronome. Stopped after $13\frac{3}{4}$ min. more exhausted than in previous experiments, but "was troubled in the last few minutes more by difficulty in co-ordinating movements of legs so as to keep time to the metronome than by actual fatigue or stiffness."

Exp. 4 (ammonium chloride). After $13\frac{1}{2}$ min. felt "slightly tired in legs but getting no worse." Stopped after $17\frac{3}{4}$ min. Perhaps getting slightly unsteady in last 2 min. but ergometer not rocking. No difficulty in keeping time with the metronome. Thought he "could have continued for another couple of minutes."

Exp. 5. After 10 min. began to get unsteady, giving the impression that the work was becoming a strain. After 12 min. legs rather tired, unsteadiness more pronounced, getting behindhand with metronome. Stopped after $15\frac{3}{4}$ min. as he was getting very shaky though he "thought he might have continued a little longer."

Exp. 6. After 7 min. began to get a little unsteady. After 9 min. more jerky in his work, looked as though the work were an effort. Stopped after 10 min. owing to fatigue and stiffness of the legs and "did not think that he could have gone on much longer." More tired in this than in the other experiments.

It therefore seems clear that the condition of the subject whilst he was doing muscular work was definitely better in those experiments in which ammonium chloride had been taken.

TABLE II.

Barometer in mm. Hg ...	421	375	347
	Pulse rate per min.		
<i>Exp. 1</i>	98	106	112
<i>Exp. 2</i>	84	88	92
<i>Exp. 3</i>	92	100	100
<i>Exp. 4</i>	80	82	86
<i>Exp. 5</i>	92	96	104
<i>Exp. 6</i>	92	108	120

The records of pulse rate afford some corroborative evidence. Table II gives the pulse rate per minute whilst the subject was sitting at rest, the counts being made about the mid-point of the periods during which

the barometric pressure remained steady at 421 and 375 mm. and about the mid-point of the period elapsing between the moment when the barometric pressure reached 347 mm. and the time when the resting alveolar samples were taken before beginning the work. The figures for the two ammonium chloride experiments are printed in italics. It will be seen that the pulse rate at rest is significantly lower in the ammonium chloride experiments.

As soon as the work stopped the subject sat down immediately and the pulse rate was at once counted. Table III shows the pulse rate (per

TABLE III. Pulse rate in successive periods of 15 sec. during the first 3 min. after stopping work.

Exp. 1	41, 41, 41, 37	—, 32, 31, 31	29, 29, 29, 28
<i>Exp. 2</i>	<i>41, 37, 33, 31</i>	<i>30, 27, 27, 26</i>	<i>26, 24, 24, 25</i>
Exp. 3	42, 40, 40, 35	35, 32, 31, 30	30, 29, 27, 27
<i>Exp. 4</i>	<i>37, 35, 34, 35</i>	<i>33, 33, 30, 29</i>	<i>28, 25, 23, 23</i>
Exp. 5	40, 35, 35, 35	33, 30, 29, 29	28, 28, 27, 27
Exp. 6	42, 39, 38, 35	31, 32, 31, 28	25, 29, 24, —

quarter minute) for each quarter of a minute during the first 3 min. after the work stopped. The pulse rate subsides more rapidly, the effect being most apparent in the first and third minutes, in the ammonium chloride experiments which are indicated by italic type.

No quantitative records of the breathing were taken. The subject did not himself appreciate any obvious difference in the degree of hyperpnœa during the work nor in the panting which persisted after the stop. Counts of the respiration rate in the first 3 min. after resuming rest showed no significant difference in any of the experiments.

All the evidence obtained in these experiments points in the same direction. The preliminary ingestion of ammonium chloride, moderate though the dose was, resulted in the following beneficial effects on exposure to a very low barometric pressure when contrasted with what was observed when no ammonium chloride was taken—a lower level of the resting alveolar CO₂ pressure (*i.e.* a greater degree of hyperpnœa) and a higher level of alveolar oxygen pressure, a lessening in the degree of cyanosis, a reduction in the pulse rate, a better condition of the subject during muscular work and an increase in the time during which he could maintain that work at a steady rate. This leaves no doubt in our minds that acclimatization to high altitudes might be hastened and assisted by taking ammonium chloride. It does not seem to us necessary to take more than a small dose to accomplish this, since we should merely be helping the kidneys at a time when they are virtually retaining acid in the body. Moreover the influence of ammonium chloride seems to be

evident for a considerable time after it has been ingested. It therefore seems to us that there is no need to advocate a dose in excess of 2 g. taken three times during the day: indeed we are inclined to think that a dose of 1 g. three times a day may prove adequate, and that the apparent benefit that Greene noted on the Kamet expedition after taking even smaller doses may well have been real.

SUMMARY.

1. The influence of the preliminary ingestion of a moderate dose of ammonium chloride on the behaviour of a person exposed to a barometric pressure of 347 mm. in a steel chamber has been investigated.

2. After treatment with ammonium chloride the subject showed a lower alveolar CO_2 pressure and a higher oxygen pressure, a lessened degree of cyanosis, a slower pulse rate and a greater ability to perform muscular work than in experiments in which no ammonium chloride had been taken.

3. The bearing of these results on the question of acclimatization to high altitudes is discussed.

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THE EFFECTS OF CHOLESTEROL AND CHOLINE ON DEPOSITION OF LIVER FAT.

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THE finding that the accumulation of fatty acids in the liver of the white rat which consistently results from the ingestion of food containing approximately 40 p.c. beef fat can be prevented by the inclusion in the diet of choline or betaine [Best and Huntsman, 1932], suggested that an investigation of the effect of these substances on fatty changes in the liver produced by other means might be profitable. The fatty degeneration found in phosphorus or chloroform poisoning is so intense that a very prolonged study will probably be required to determine the effect, if any, of choline. In a consideration of fatty changes in conditions which might be of more physiological importance, several possibilities presented themselves. Two of these have been investigated—pregnancy in rabbits and cholesterol feeding in rats. It has been stated that the fatty acid content of the rabbit's liver is appreciably increased during the late stages of pregnancy. With the intention of determining the effect of choline on this change the total fatty acids have been estimated in the liver of pregnant rabbits at or near term. The results of this study, which was carried out in large part by our colleague Dr D. L. MacLean, failed to demonstrate a consistent increase in the amount of liver fat even when the pregnant animals received a diet high in fat. We have been more fortunate, however, in producing fatty changes in experiments in which cholesterol has been added to a diet which alone did not produce an appreciable increase in the liver fat of white rats.

The fact that the addition of small amounts of cholesterol to the diet of rats produces an increase in liver fat has been established by Yuasa [1928], who studied the liver by histological and chemical methods in several species but did not investigate chemically the fatty acid content of the organ in this species. W. M. Sperry has very kindly informed us that he has noted fatty livers in cholesterol-fed rats. We have had no difficulty in confirming these results and are reporting in this communica-

tion the action of choline and betaine, both of which prevent the deposition of liver fat attributable to ingested cholesterol under the conditions of our experiments.

EXPERIMENTAL PROCEDURE.

White rats of the Wistar strain weighing between 150 and 250 g. were placed in individual cages and given a mixed grain diet consisting of equal parts of whole cracked wheat, rolled oats, corn meal and 2.5 p.c. bone meal. The cholesterol was dissolved in an hydrogenated vegetable oil (Crisco), which had been previously melted over a water bath, and was added with thorough mixing to the grain diet. The hydrogenated oil, which has an iodine number of approximately 63, formed 20 p.c. of the diet. The various groups into which the rats were divided were as similar as possible. The amount of food consumed daily by each rat was carefully determined and from this the amount of cholesterol ingested was calculated. When choline or betaine was given it was added to the diet in aqueous solution and thoroughly mixed. The amounts of cholesterol and of choline ingested are given in Table I. After approximately 1 month on

TABLE I. Summary.

No. of rats	Length of exp. days	Av. change in wt. g.	Av. fat eaten per diem g.	Av. fat excreted per diem g.	Iodine No. of fat in faeces	Av. cholesterol eaten per diem g.	Av. choline* eaten per diem g.	Fatty acid in liver p.c.	Iodine No.
14	31	-23	1.57	0.10	41	—	—	5.46	107
9	33	-11	1.60	0.11	43	—	0.07	5.44	116
23	27	-20	1.97	—	—	0.19	—	8.79	—
15	31	-19	1.68	0.20	45	0.13	—	10.56	95
14	28	-5	1.97	0.18	48	0.09	—	10.75	95
17	28	-7	1.97	0.19	41	0.19	—	11.60†	91
13	31	-11	1.76	0.19	46	0.14	0.07	8.21	95
13	31	-19	1.98	0.13	42	0.10	0.07	7.51	107
29	30	-12	1.98	0.23	49	0.19	0.10	6.44	108
15	28	-21	1.99	0.17	47	0.19	0.19	4.50†	103
19	27	-24	1.97	—	—	0.19	0.19	3.99	—
Betaine									
14	27	-22	2.00	0.16	—	0.20	0.33	3.36	—

* Choline hydrochloride B.D.H. was used and the dosage is expressed in terms of the base.

† The individual values in these two series are listed in Table II.

these diets the rats were killed and the livers removed immediately for estimation of total fatty acids, using the Leathes and Raper modification of Liebermann's saponification method. The iodine number was determined by the Rosenmund-Kuhnhehn procedure [1923].

RESULTS AND DISCUSSION.

The significant results are summarized in Table I. Rats fed on the basal diet have from 3 to 5 p.c. liver fat. In a series of fourteen rats the basal diet plus the hydrogenated oil produced a very slight increase in liver fat. Choline added to the basal diet plus the hydrogenated oil did not appreciably change the amount. In the four series of animals which received cholesterol and no choline the average fatty acid content was just over 10 p.c. It is interesting that 70 mg. of choline daily were inadequate completely to prevent the deposition of fat attributable to cholesterol, but would certainly, on the basis of previous results, have been sufficient to control the fatty changes produced by a diet containing 40 p.c. beef fat. However, when 190 mg. of choline were provided the average liver fat did not exceed the value found in the control group. Betaine also prevented, in a very satisfactory manner, the effect of cholesterol on liver fat. The figures in Table I also show that the fat excretion was approximately the same in the experiments with and without choline.

Since the average figure for liver fat might be misleading to other investigators the fatty acid values of the individual livers for a cholesterol and a cholesterol-choline series are given in Table II.

TABLE II. Estimations of fat in liver (fatty acids p.c., and Iodine No.) in individual rats receiving:

A. Cholesterol <i>without</i> choline				B. Cholesterol <i>with</i> choline			
Fatty acids p.c.	Iodine No.	Fatty acids p.c.	Iodine No.	Fatty acids p.c.	Iodine No.	Fatty acids p.c.	Iodine No.
7.02	97	11.41	90	4.02	110	4.87	104
17.00	92	8.65	95	4.05	99	4.06	108
11.58	90	16.10	88	3.81	101	4.69	100
16.81	91	4.93	101	3.81	—	6.68	96
6.30	—	16.34	85	8.15	86	2.80	—
10.58	92	14.12	88	4.82	102	4.06	111
6.84	96	10.20	96	4.36	101	2.94	99
13.05	81	17.51	84	4.42	120		
8.69	94						

Choline or betaine produced no appreciable change in the fatty acid content of the kidney or stomach tissue of the cholesterol-fed animals.

It appears to be a foregone conclusion that orally administered lecithine would prevent the accumulation of liver fat produced by cholesterol in the same way that choline has been shown to do. This finding should provide clearer evidence for a "cholesterol-lecithine antagonism" than several of the frequently quoted results [Degkwitz, 1931].

Although choline prevents deposition of liver fat in cholesterol-fed animals there is no evidence as yet that this base is concerned in cholesterol metabolism, and in this series of experiments cholesterol was chosen merely because of its effect on deposition of liver fat. While the results thus far obtained suggest that the unsaponifiable fraction of liver fat may be less in the animals receiving choline than in the group on cholesterol alone, further experiments are required to settle the point. The results of this preliminary study suggest that investigation of the effect of choline on the cholesterol content of tissues under various conditions, as well as further work on liver fat in cholesterol-fed animals, may be profitable.

SUMMARY.

The deposition of fat in the rat's liver produced by feeding cholesterol can be prevented by adding choline or betaine to the diet.

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OBSERVATIONS ON THE RETINAL ACTION
POTENTIAL WITH ESPECIAL REFERENCE
TO THE RESPONSE TO INTERMITTENT
STIMULATION.

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IN the publications of the early workers on retinal action currents, observations on the response to intermittent light are often reported. The first to undertake such experiments were, we believe, Kühne and Steiner [1880], who produced intermittent light by rhythmically compressing the rubber tube leading to their gas flame. But, apart from the finding that with this mode of stimulation the response also became rhythmic below a certain frequency of alternation, no really significant contributions were made to the problem until Piper in 1911 investigated the reactions of various types of vertebrate retinae to an intermittent stimulus of given strength. He observed several interesting facts which, as occasion arises, will be referred to below in connection with our own observations. Some general conclusions based on Piper's work still summarize the state of knowledge. The retinal response to intermittent light consists of undulations during the slow phase of the retinal action potential (*c*-wave), which disappear above a certain frequency of stimulation. This critical frequency is low in eyes containing mainly rods (cat), and very much higher in eyes in which cones predominate (pigeon). Where the retinal action current shows a well-developed initial negative *a*-wave and a marked off-effect (*e.g.* frog), these two appear to be among the factors determining the troughs and crests of the waves. Some observations of a similar character on the eyes of fishes were made by Day [1915] in Piper's laboratory.

Since that time the retinal reaction to intermittent light has hardly been studied at all by direct methods. Renqvist [1924] noted that

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flashes superimposed upon steady illumination of the frog's eye gave rise to small *a*- and *b*-waves on the background of the slow *c*-wave. Sachs [1929], recording from his own eye, found that intermittent potential waves were accompanied by a sensation of flicker, whereas a smooth potential curve corresponded to the sensation of continuous light. Adrian and Matthews [1928] recorded the impulses in the optic nerve of the conger eel and found that below a certain frequency, increasing with the area and intensity of the stimulus, the discharge waxed and waned at the same rhythm as the stimulation, but that above that frequency it was represented by a steady stream of impulses such as is given by continuous illumination.

Several problems have thus been raised which are of importance both from the point of view of the processes in the retina and in connection with the sensory phenomena of flicker and fusion. The experimental work presented below is concerned only with the retinal action potential and not with flicker. When the terms "fusion" and "fusion frequency" are used, they refer to the point at which the intermittent potential waves cease. Much remains to be done before a complete understanding of what occurs in the retina can be reached. We have attempted no more than a general survey of the ground in the light of recent additions to knowledge of retinal physiology. In particular, the new evidence [Granit, 1933] regarding the three components of the action-current complex necessitates a study of how responses deficient in one or other of these components behave when the stimulus is intermittent. In order to be in a position, however, to discuss the effects of repetitive stimulation, it seemed desirable first to investigate the responses to single flashes. Part I of this paper contains the results of this study.

METHOD.

Records have been obtained from some 25 cats, decerebrated 2 hours before beginning the observations. During this interval the preparation was shut up in a box so as to ensure complete dark-adaptation. The pupil was, in all preparations, widely dilated. The animal technique and the methods of stimulating and recording were the same as those described in a previous paper [Granit, 1933]. The only modification of the apparatus for purposes of this research has been the introduction of a sectored metal disc which could be rotated in a plane where the cross-section of the stimulating beam was narrow. The disc was driven by an electric motor, the speed of which could be controlled by a sliding resistance. Three discs

were employed, with open sectors of 180° , 90° , and 45° respectively. The opaque sectors in each were of the same size as the open sectors. The third disc thus had four open and four opaque sectors.

The circular ground-glass sheet constituting the stimulus object was 46 mm. in diameter and was mounted 370 mm. from the cat's eye where it subtended 7° . The intensity of the light falling upon it was varied by inserting Wratten neutral-tint filters, with transmission factors of 0.5, 0.1, 0.01, and 0.001, in the beam. At full intensity the brightness of the ground glass was about 11 millilamberts. The area of the stimulus was varied by mounting an iris diaphragm just in front of the ground glass. Since we do not know how sharp was the image formed on the retina, it is impossible to state confidently what precise effect on the area of this image a known change in the area of the stimulus object would produce. On ophthalmoscopic observation, the decerebrate cat's eye appears to be approximately emmetropic. The duration of the stimulus was controlled by a Compur photographic shutter placed where the beam was narrow.

To obtain the "fusion frequency" with intermittent stimulation, the motor driving the sectorized disc was slowed down from a high speed, or, less frequently, speeded up from rest. The point at which the string began to oscillate or became steady respectively was later determined from the tracing. Frequencies of alternation are always expressed in flashes per second.

The periods during which the eye was exposed to light were directly photographed on the moving strip of sensitized paper. The duration of each flash was thus readily measured with the aid of the time marker (Rayleigh wheel) simultaneously registering intervals of 20σ . The slight spread, or "irradiation," of photochemical effect on the paper could be accurately estimated by comparison of the apparent durations of the (actually equal) light and dark phases, as recorded by the signal when the motor was running steadily. Judged by this criterion, our measurements of latent period, always made with the paper moving at maximum velocity in the camera, are 3σ too long.

The use of valve amplification of the retinal action potential has enabled us to keep the string of the small permanent magnet string galvanometer (Edelmann's model) tense enough to follow rapid changes accurately and to give reliable information about the latent period. Variation of such factors as area and intensity of the stimulus could then be studied over a certain range. But the more sensitive the recording apparatus, the more difficult it is to obtain a base line sufficiently steady to record oscillatory responses of small amplitude from an eye *in situ*.

Slight movements of the eyelids and of the external ocular muscles are liable to confuse the picture. Moreover, the eye of the decerebrate cat, even when thoroughly dark-adapted, is an unsuitable preparation for investigation of the effects of either greatly diminished area or greatly diminished intensity of stimuli. When using low intensities of stimulation, we have rarely found it possible to work with initial *b*-wave deflections of less than 5 mm. Illuminations lower than about 0.01 ml. have only occasionally been used.

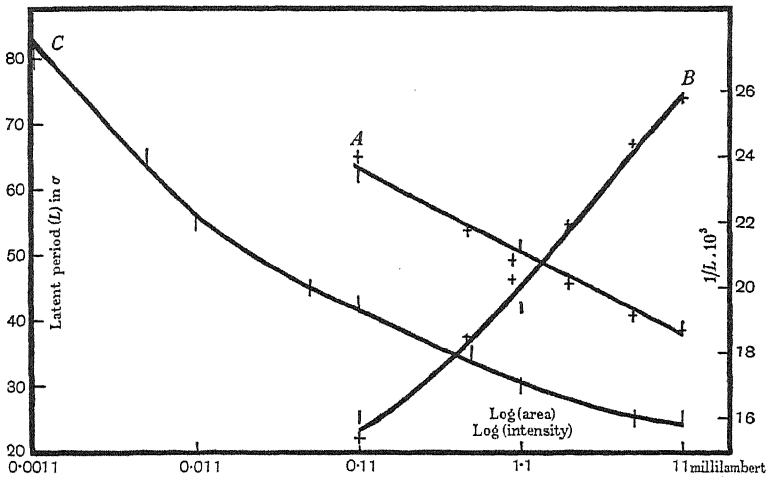
I. CONTINUOUS STIMULATION AND SINGLE FLASHES.

The electrical response of the retina may be affected by the area, by the intensity, and by the duration of the stimulus that evokes it. We have investigated the influence of each of these factors separately. The results may be stated very briefly because, in the main, they merely confirm and extend the conclusions of previous workers. Shortening of the latent period with increasing intensity of stimulus has been repeatedly observed [Einthoven and Jolly, 1908; Ishihara, 1906; Adrian and Matthews, 1927 *a* and *b*; Sachs, 1929; Granit, 1932]. The similar effect of increasing the area of a stimulus of constant intensity has been studied by Ishihara [1906], Adrian and Matthews [1927 *a* and *b*], and Granit [1933].

In Text-fig. 1, curve *A* represents the relation found in one experiment between latent period preceding the *b*-wave and either the area (crosses) or the intensity (vertical strokes) of the stimulus. Each value plotted is the average of five readings. With a constant area of 1660 sq. mm. placed 370 mm. from the eye, the intensity was first reduced to 0.1 and 0.01 of its original value (11 ml.). The filters were then removed, and the area reduced in several stages, again to 0.01 of its initial extent. The abscissæ are log area and log intensity plotted on the same scale, the lowest point on the curve being the common starting point for both sets of observations. In this experiment and within these limits, area and intensity evidently influence the latent period to precisely the same degree. They do not, however, invariably do so. Curve *B* is plotted to the same abscissæ, but the ordinates are the reciprocals of the latent periods in *A*. Curve *C* shows the results of another experiment in which a stimulus of the same area was placed about 70 mm. from the eye. The higher potential developed enabled measurements to be made over a ten-thousandfold range in intensity. Each point is a single reading. The latent period at the lowest intensity is somewhat uncertain on account of the gradual development of action potential. Similar results have on several occasions been

obtained with this large stimulus. The points do not lie on a straight line over the whole range. When the reciprocal of the latent period is plotted against log intensity, an S-shaped curve is obtained.

The amount of potential, measured by the height of the *b*-wave, and the rate at which it develops have been found to vary as would be expected both with the intensity and with the area of the stimulus. The findings in this connection have not been analysed in detail, but are in rough agreement with previous work.



Text-fig. 1. The ordinate scale on the left gives the latent period of the retinal response for curves *A* and *C* in σ . That on the right gives the reciprocal of the latent period for curve *B*. Abscissæ are log brightness in millilamberts and log area in arbitrary units. For explanation, see text.

The duration of the stimulus is in our experience devoid of influence on the latent period. The shortest flashes we could obtain have been about 4σ in length. These have been tried on a number of different preparations, and the latent period preceding movement of the string has always been the same as that for prolonged stimuli. Thus, in Plate I, fig. 2, the latent period is 30σ both for a flash of 4σ and for one of 110σ . At very low intensities measurable records could not often be obtained with such brief exposures. A brightness of 1.1 ml. has sometimes been used. The results are then the same as with more intense stimuli. The latent period for a stimulus of 0.11 ml. is also unchanged by shortening the flash to 40σ . Briefer exposures are impracticable with so feeble a stimulus.

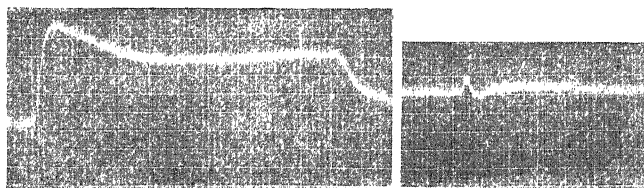


Fig. 1.

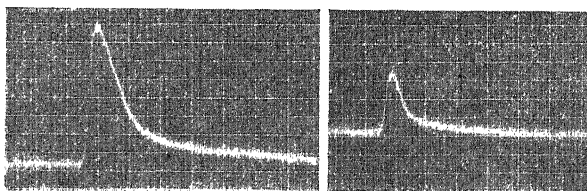


Fig. 2

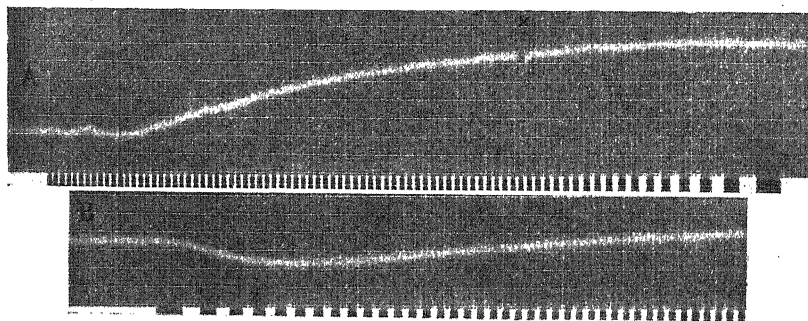


Fig. 3.

Plate I. Fig. 1. Records showing the effects of continued stimulation and of a flash lasting about 4σ , both of intensity 1.1 ml. The signal at the bottom indicates the beginning and the end of the stimulation. The thin vertical strokes are 20σ , and the thick 100σ , apart. The interval between the horizontal rulings is 5 mm.

Fig. 2. Responses to two flashes, one lasting about 110σ and continuing beyond the peak of the b -wave, the other lasting about 4σ . Brightness of stimulus 11 ml. Both have the same latent period.

Fig. 3. Both records show responses to intermittent light of intensity 11 ml. In *A* the carotid artery has been ligated. P II is in consequence very small (minute b -wave). The cross marks an artefact produced by throwing a high resistance into the circuit of the motor driving the sectorised disc. In *B* P II has been removed by ether, leaving only P I and P III. There is no trace of ripples.

The amount of potential developed is, however, generally diminished by making the flashes shorter (Plate I, fig. 2). With the more intense stimuli this was not always so. The *b*-wave might then be as high with a flash of 4σ as with a longer stimulus. But as a rule the *b*-wave became progressively smaller as the flash was shortened below 20σ (cf. Hartline [1928], on the eyes of arthropods). Piper [1911], who used strong stimuli, found that "within wide limits" the *b*-wave was independent of the time of stimulation.

In one experiment, illustrated in Plate I, fig. 1, the form of the curve resulting from very short flashes differed from that usually seen. To exclude chance variations in the base line, it was photographed several times, always with the same result. *A* is the response to a long-lasting stimulus of 1.1 ml. *B* is a flash of about 4σ . It shows a small initial negative wave, not visible in *A*, followed by the *b*-wave, a small negative deflection, and a secondary rise. It is reminiscent of the recurrent sensory images elicited by short flashes. It may indicate that for unknown reasons conditions were favourable in this experiment for some complicated kind of interaction between the processes P II and P III [Granit, 1933].

Discussion. It is not the purpose of this paper to investigate the relation between quantity of light and height (or area) of the curve of potential. On the other hand, the absence of any change in the latent period as a result of using the briefest flashes was so unexpected a finding that some discussion of it is demanded.

The effect of varying area or intensity is easily detected and is in no way surprising. At low intensities it is probably exaggerated artificially by the gradual ascent of the rise of potential. But Adrian and Matthews [1927 *b*] found that below a certain critical duration, the latent period preceding the discharge of impulses in the conger eel's optic nerve was lengthened by shortening the flash. It is probable that at the high intensities we were using 4σ is longer than the critical duration for the dark-adapted cat's eye, and that lengthening of the latent period would have been observed had we been able to obtain still shorter flashes. At low intensities too little potential may have been elicited with flashes below the critical duration. But it is also possible, though less probable in view of the finding by Adrian and Matthews of a constant retinal nerve interval in frogs and conger eels, that the latent period of the discharge in the optic nerve is not directly related to that of the *b*-wave. Our results cannot be explained by assuming that the initial negative wave disappears with short flashes and thereby allows the rise of the

b-wave to be determined at an earlier stage. We have several high intensity curves in which the *a*-wave is to all appearance unchanged when the flash is shortened to 4σ . It is worth emphasizing that although the latent period would probably be affected by the length of exposure with very short flashes, this phenomenon does not appear comparable with the effects of area and intensity.

From the effects of diminishing area and intensity, and from those of narcotization and asphyxia [Granit, 1933], it might be inferred that every reduction in the height of the *b*-wave is accompanied by a lengthening of the latent period. The experiments with flashes, however, make it certain that no such correlation can be established.

II. INTERMITTENT STIMULATION.

Plate II, tracing *A*, shows the response to a stimulus of full intensity, intermitted at a constant rate of 10·8 per second. It will be noted that the well-developed *b*-wave, characteristic of intense stimulation, tends to take its own course and does not show oscillations in response to the first few flashes. Later, oscillations become obvious, but the earlier of them are smaller than those set up after the lapse of 1 sec. These facts were also observed by Piper [1911]. Finally a semi-stationary state is reached in which ripples of unvarying size are superimposed on the background of the *c*-wave. The oscillations show no sign of diminishing in amplitude towards the end of the 5 sec. for which the stimulus in one experiment was applied. The latent period from the beginning of the flash to the beginning of the upstroke also remains constant. The oscillations are remarkably symmetrical provided the frequency of repetition of the stimuli is high enough. In *A* the symmetry is to some extent caused by the slackness of the string. In *B*, however, the string is tense enough to justify the conclusion that the rise and the fall of the action potential itself are much alike in steepness. The upstrokes are perhaps slightly more steep than the downstrokes.

When the *b*-wave is smaller (as, for instance, with less intense stimuli), or when the frequency is low, ripples corresponding with the rate of stimulation appear in the records from the very beginning. It is only large *b*-waves that succeed in masking more or less completely the initial rhythm (see Plate II, *B* and *F*). In some tracings taken with stimuli of 0·011 ml. the ripples are actually larger and rise more steeply than the initial *b*-wave elicited by a continuous stimulus of the same intensity.

When the frequency is increased, the oscillations become smaller until no trace of them is to be seen (Plate II, *B*, *C*, and *D*). The fusion frequency can be determined from records like Plate II, *E* and *F*, during

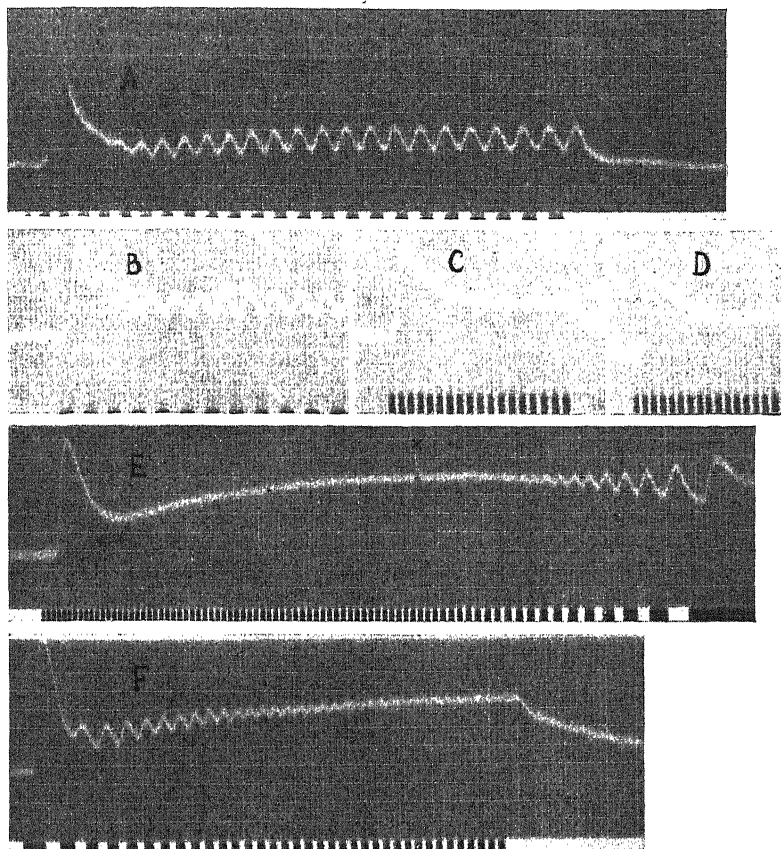


Plate II. Series of responses to intermittent light (intensity 11 ml.). *A*, string slack. Constant rate of stimulation of 10.8 flashes per second. The *c*-wave is unusually small. *B*, *C*, and *D* are from another experiment. They show the effect of increasing the frequency of alternation. *E*, from another experiment, is typical of the response when the motor is slowed down. The cross indicates an artefact as in Plate I, fig. 3. *F*, from another experiment, illustrates the effect of speeding up the motor. Note the *c*-waves in *E* and *F*.

which the motor was slowed down and speeded up respectively. The former method, by which most of our results have been obtained, gives slightly lower values (2–3 flashes per second) than the latter. The highest

fusion frequency that we have found for the dark-adapted cat's eye is 32 with a stimulus of about 11 ml. In most cats the maximum frequency is probably not higher than 25 or 30 [cf. Piper, 1911]. Considering that in our experiments the pupil has been widely dilated, and that the animal is nocturnal and thoroughly dark-adapted, we have every reason for believing that a stimulus of the order of magnitude of 10 ml. or 100 m.-c. is physiologically maximal.

Control experiments.

It might be expected that the apparent fusion frequency in the tracing would vary with the intrinsic frequency and sensitivity of the string. The slackest string used has been one taking about 50σ to reach its full deflection when a constant current is passed through it. About the same time is occupied by the rising phase of the fastest *b*-waves of the cat's retinal action potential with stimuli of our maximal intensity.

TABLE I. Fusion frequency of intermittent potential waves for a stimulus of constant strength, 11 ml. The values were obtained with the string at three different tensions. At each tension the sensitivity was varied by shunting the string through different resistances. The sensitivity is indicated by the deflection in mm. of the *b*-wave of the retinal action potential, which, throughout the experiment, was 0.58 millivolt. The string was unshunted only when maximally tightened (rising to maximal value in 2.5σ); in the other cases the damping is considerable. String resistance 3100 ohms.

String aperiodic, rising in 57σ		String just periodic, rising in 5σ		String periodic rising in 2.5σ	
Height of <i>b</i> -wave	Fusion frequency	Height of <i>b</i> -wave	Fusion frequency	Height of <i>b</i> -wave	Fusion frequency
21	24	9	22	30	23
21	21	11.5	24	30	22
39	25	18	25	30	23
40	23	18	22	—	—
—	—	57	24	—	—

Table I contains the results of one of the experiments made to test the influence of these factors. It is evident that considerable variation in string tension does not affect the fusion frequency. The values of the fusion frequency are typical of those obtained under the same standard conditions of stimulation in a large number of other experiments. The variations in the value are also typical. A high degree of accuracy in the determination of the exact point of fusion is unattainable even when, as in this and most other series, the motor is slowed down for every observation. The unlikely possibility is not altogether excluded that the variations may arise in part from the fact that in different records the fusion point occurs at different intervals after the onset of stimulation.

Just as the retinal action potential cannot be satisfactorily recorded when the stimulus, although easily visible with the human eye, is feeble, so no doubt there is a limit to the smallness of the oscillations that can be detected in the tracings. The stability of the base line varies inversely with the sensitivity of the recording apparatus. In other words, it is by no means certain that the retinal processes entirely lose their oscillatory character when the frequency of stimulation is above what is here called the fusion point. The true fusion frequency may be higher. Nevertheless, variations in the fusion frequency caused by changes in area and intensity of the stimulus, as determined from our records, are of real significance and must correspond with similar variations in the true fusion frequency. What the control experiments show is that our criterion of fusion frequency, even if, in a sense, a fictitious one, is unaffected by considerable variations in the tension and the sensitivity of the string.

Isolation of the process responsible for the oscillations.

The complex retinal action currents have recently been analysed into three components known as P I, P II, and P III [Granit, 1933]. The positive P I rises and falls slowly, and is principally responsible for the secondary rise or *c*-wave with strong stimuli. P II causes the *b*-wave and part of the *c*-wave at high intensities. With weak excitation the electrical response gives a maintained positive deflection of simple form, almost entirely made up of P II. P III is negative. To it alone is due the *a*-wave. By methods described elsewhere, we have removed one or other of these processes in order to determine their respective rôles in the production of the oscillations.

P I is readily abolished by giving the preparation ether to inhale. The ripples persist and the fusion frequency is unaffected.

P II is very minute as a result of asphyxia in Plate I, fig. 3 A. Although the intensity is 11 ml. and the *c*-wave is well developed, there is only the merest trace of rippling at the end of the record. Complete removal of P II causes total disappearance of oscillations, though no doubt the *c*-wave might be made to wax and wane if the periods of light and darkness were each of some seconds' duration. But this would clearly be a different phenomenon.

In Plate I, fig. 3 B, both P I and P II have been largely removed by a high concentration of ether. The remaining negative deflection (P III) traces a smooth curve.

Hence it is clear that P II is the component chiefly responsible for the ripples in Plate II. Moreover, at low intensities, P II is manifested in an

almost pure form [Granit, 1933]. Oscillations are then readily recorded, and are so large that they cannot possibly be ascribed to the tiny effects of P I and P III. The conclusion is in keeping with the finding of Adrian and Matthews [1928] that the optic nerve discharge waxes and wanes with intermittent stimulation, coupled with Granit's inability to show that any component other than P II is concerned with the discharge of impulses [1933]. It also harmonizes with Renqvist's observation [1924] on the frog's eye (where α -waves are large) that flashes superimposed upon a steady stimulus give rise to small α - and b -waves without affecting the slow rise of the c -wave.

The possibility that P II and P III interact, when present together, is not excluded. No means is available of removing P III at high intensities, leaving P II isolated for study. Evidence will be furnished below that such interaction does in fact take place, but we cannot say whether it has any effect on the fusion frequency. It may be that the fusion point and the shape of the individual ripples would be altered were P II released from the modifying influence of P III.

Factors influencing fusion frequency.

In the upper part of Table II figures are given showing how, in one of many similar experiments, the fusion frequency, determined as described above by slowing down the motor, varies with the brightness and

TABLE II. Effects of variation in area and intensity on the fusion frequency and the latent period of the intermittent potential waves of the retinal action potential. The values are averages of 3-4 measurements for the fusion frequency and of 10 or more for the latent period. Distance to cat's eye 370 mm. Maximal area of 1660 sq. mm. represented by circular disc with a radius of 23 mm. D is the duration in σ of the flashes at the fusion point. L is the latent period of the intermittent waves in σ .

Experiments with constant area of 1660 sq. mm. Brightness varied				Experiments with constant brightness of 11 ml. Area varied			
Brightness	11	1.1	0.11	Area 1660	473	165	16.7
Fusion frequency	19.3	16.8	14.8	19.3	18.0	15.8	13.6
D ...	26.0	29.8	33.8	26.0	27.8	31.6	36.8
L ...	60	76	95	60	65	71	84
D expressed as per- centage of L	43	39	36	43	43	45	44

with the area of the stimulating object. The range of variation in each series is a hundredfold. As a rule a given increase in intensity has a greater effect than a corresponding increase in area. If the fusion frequencies are plotted against log brightness or log area, they lie on approximately

straight lines. It is obvious from Table I that great accuracy cannot be expected in such experiments.

The series do not cover a wide enough range to permit the definite formulation of a general law relating fusion point and brightness or area, such as has been found to hold for sensory fusion of flickering stimuli. Adrian and Matthews [1928] similarly noted an increase in the fusion frequency for the optic nerve, as defined by the cessation of rhythmical waxing and waning of impulses, when either the area or the intensity of the stimulus was increased.

The lower part of Table II illustrates an empirical rule relating the fusion frequency with the latent period of the ripples in the curve of retinal action potential. By "latent period" we mean the interval between the beginning of a flash and its corresponding ripple. Each value given for the latent period is the mean of a large number of determinations and is therefore a fairly reliable figure. Many determinations can be made from every record, since the length of the latent period appears to be unaffected both by the frequency of intermission and, at any rate within our limits, by the total length of the exposure. (It will be explained in a later section, however, that it is not necessarily the same as the latent period for a continuous light.) The product of the latent period and the fusion frequency is approximately constant. This is equivalent to saying that, at the point of fusion, the duration of the flash (one-half the reciprocal of the fusion frequency) is a fixed fraction of the latent period. The last line of the table expresses this fraction as a percentage. In view of the difficulty of accurately measuring the fusion frequency, the variations in this figure cannot but be regarded as very small.

Results obtained with preparations other than the one from which Table II is constructed give much the same value. In all, 50 independent determinations of the fraction have been made, each representing the average of all the values obtained with the same area and same intensity on any given day with the motor slowing down. The arithmetic mean of these is 44 p.c., with a mean variation of 4.3 (= 9.8 p.c. of 44). When the flashes at the fusion point are so frequent as this, it is evident that each occurs shortly before any potential can have been developed by the one immediately preceding it. There is thus scope for interaction between the retinal processes set up by successive flashes.

The rule should not be regarded as more than a convenient first approximation to a formula relating length of flash at the point of fusion and latent period. The range of variation on which it is based is insufficient to justify far-reaching conclusions. It may well be that over a wider

range some systematic deviation would be found, but within the limits that we have investigated it appears to be true that the fusion frequency varies inversely as the latent period of the oscillations.

Rapidly intermittent stimulation and the Talbot-Plateau law.

According to the Talbot-Plateau law, whenever intermittent stimuli follow one another with sufficient rapidity to give rise to a sensation of continuous light of uniform brightness, the apparent brightness is that which would have arisen had the amount of light intermittently reaching the retina been uniformly distributed over the whole period of stimulation. The law has repeatedly been verified over a wide range of intensities and over a wide range of variation in the relative lengths of the bright and dark intervals. Only at very high and at very low intensities have exceptions been reported. For all ordinary conditions the law is so reliable that many workers regard the episcotister as the method of choice for producing accurately known variations in the physical intensity of illumination. It becomes of interest, therefore, to know whether a similar relation holds when the retinal action potential, instead of the subjective brightness, is investigated.

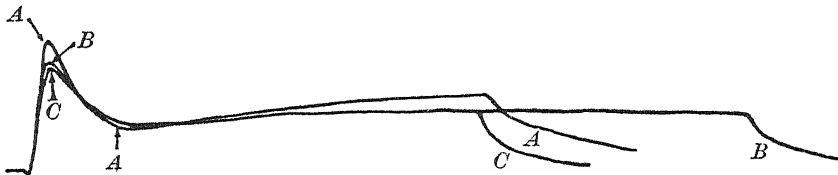
We have made no systematic series of records, but in the course of other enquiries we have compared steady tracings from retinæ intermittently illuminated at a fast speed of alternation, light and darkness being of equal duration, with others from the same retinæ continuously illuminated from the same source but with the intensity reduced to a half by means of a Wratten neutral-tint filter. That the fused curve from the intermittently illuminated retina is lower than that from the retina continuously illuminated at full intensity is clear from experiments in which the motor has been speeded up from rest with an open sector between the source of light and the eye. That its height is identical, within the limits of experimental error, with that from the retina continuously illuminated at half-intensity is shown in Text-fig. 2. So far, then, as the potential developed is concerned, a Talbot-Plateau relation seems to hold under these conditions. The latent period, however, may be different in the two cases and must be considered separately.

Since the latent period of the initial *b*-wave is the same for short flashes as for continued illumination, it might be expected that it would also be the same for stimuli rapidly alternating with darkness. This indeed is often so. But not uncommonly, as shown in Table III, the intermittent stimulus requires much longer than the continuous to produce any effect. Moreover, it will be noticed that the latent period with the continuous

TABLE III. Comparison between initial latent period in σ for continuous and for rapidly intermittent stimulation.

Brightness in ml.	A, continuous stimulus	B, intermittent stimulus	Ratio B/A
11	37	73	2
1.1	40	69	1.7
0.11	50	65	1.3

stimulus progressively shortens as the intensity is increased. Yet, in the same experiment, when the stimulus alternates at 30–40 flashes per second, a converse relation holds between latent period and intensity. And the higher the intensity, the more does the length of the latent period differ from that with continuous illumination. As has been stated above, the phenomenon cannot always be demonstrated, but it is clearly of great interest and must later be discussed in connection with our other findings.



Text-fig. 2. A is the response to continuous illumination at 11 ml., C to 5.5 ml. B is the response to an intermittent stimulus (40 flashes per second) of 11 ml. The rotating disc gives equal periods of light and darkness. The *b*-wave of B is slightly larger than the corresponding wave of C, although the two curves coincide in their further course.

At the moment it need only be pointed out that the initial latent period for a rapidly intermittent stimulus is often longer than that for a steady stimulus of half the intensity. The two forms of stimulation are therefore not equivalent in all respects, and a Talbot-Plateau relationship cannot be established for the latent period.

So far we have only considered the course of events with a rapidly alternating stimulus of considerable duration. This is perhaps the only condition that Talbot and Plateau and most subsequent investigators of their law have had in mind. But, with very short exposures of a bright intermittent light, when the rate of intermission is only just high enough to cause under these conditions a steady sensation, it has been found [Granit and Hammond, 1931] that the subjective brightness is greater than the law would allow. This is entirely in keeping with the fact recorded above and by Piper [1911], and illustrated in Plate II A and C, that at rates of alternation insufficient to produce smooth tracings, the initial *b*-wave may nevertheless reveal no trace of discontinuity until several

flashes have occurred. In these circumstances the *b*-wave is larger than at higher rates. The Talbot-Plateau law, both as regards sensation and as regards retinal action potential, only holds when the rate of intermission is fast enough to cause fusion for a prolonged period.

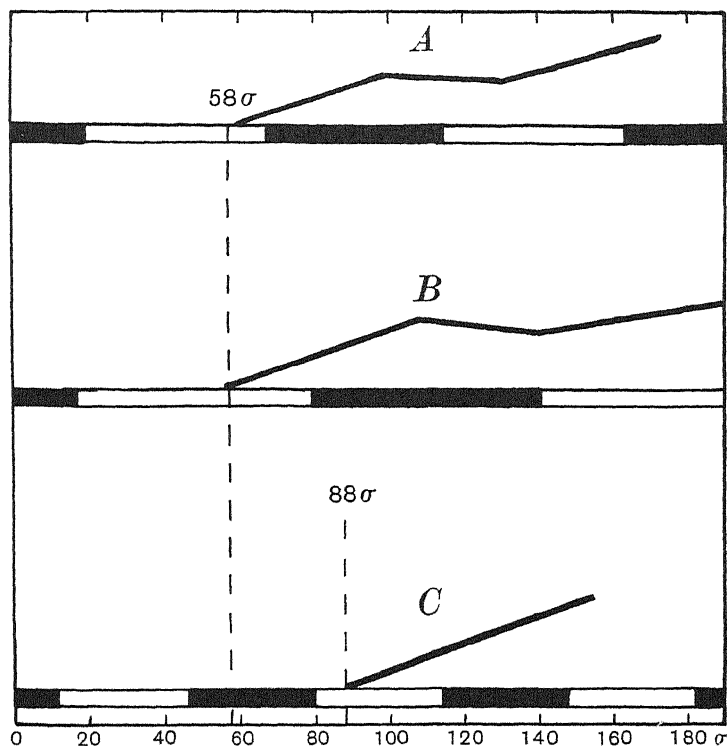
The latent period preceding the oscillations.

When we have followed the usual procedure of slowing down the motor from a speed much above the fusion frequency, it has been our almost invariable experience that the latent period preceding the upstroke of the waves in the later part of the tracing has been longer than that preceding the initial *b*-wave. Sometimes it is twice as long. And it was explained in a previous section that the latent period of the initial *b*-wave itself is commonly longer than that for a steady stimulus of the same intensity. The initial *b*-wave, it is true, rises from a steady base line, whereas the ripples rise from the downstrokes of the preceding ripples. Instrumental inertia cannot, however, account for the phenomenon, for it can be made negligible by tightening the string and at the same time increasing the amplification. Nor can the long latent period be ascribed to fatigue, since the latent period of oscillations remains approximately constant when a regularly intermittent stimulus is continued for as long as 5 sec.

Some light is thrown upon the factors responsible for lengthening the latent period by an experiment illustrated diagrammatically in Text-fig. 3. In this the motor was driven at a slow speed from the beginning of each observation. The rising phase of the *b*-wave, occurring sometimes in two stages with such a slow rate of stimulation, is plotted against time in σ . The curves *A*, *B*, and *C* are all for the same intensity of stimulation and end at the highest point of the *b*-wave. In *A* and *B* the latent period (unusually long for a stimulus of 11 ml.) is about 58σ . In *C*, however, the latent period is 88σ and the string has not begun to move until some time after the second flash has been delivered. The *b*-wave here rises in a single step to the same height as is attained at the second step in *A* and *B*. It is evident that the first flash alone in *C* would have elicited a smaller *b*-wave, and therefore that the rise represents the sum of the effects of two flashes. But why was the beginning of the rise delayed for 30σ as compared with *A* and *B*?

The second flash occurred just before the expiration of the normal latent period of 58σ following the first. It seems that the delivery of the second flash has delayed the appearance of the *b*-wave caused by the

first. For, although the first flash is briefer in *C* than in *A* or *B*, it was shown in Part I that the latent period is independent of the duration of the flash. The cause of the delay will be dealt with more fully in the discussion. Once the delay is over the effect of the second flash summates with that of the first. This is shown not only by the height of the resulting *b*-wave but also by the fact that the *b*-wave commences 42σ after the beginning of the second flash, which, if alone responsible for it, would have



Text-fig. 3. For explanation, see text.

been followed by a latent period of 58σ . The whole experiment is a good illustration of how complicated may be the effects of repetitive stimulation.

DISCUSSION.

When discussing the sensory phenomena of intermittent visual stimuli and the Talbot-Plateau law, many writers [*e.g.* Grünbaum, 1898; Parsons, 1924] have concluded that the individual flashes of light

cannot be considered as merely reinforcing one another's action. Some factor, or factors, other than summation of the excitatory effects of the successive periods of illumination must come into play. The observations which have been recorded above fully confirm this view. Some might hope to find a solution to the problem of this additional influence by recourse to the possible stimulating effect of darkness following light. We propose, however, to confine our attention to certain other considerations which are based on a strong experimental foundation.

It has been shown that the latent period preceding the retinal potential changes may be longer with rapidly intermittent than with steady illumination, and that this is especially likely to be found with intense stimuli. With alternations not rapid enough to cause a steady response, it has further been shown that the latent period of the ripples is approximately constant, but is often very much longer than that of the initial *b*-wave with a fast motor. Lastly, it has been shown that the response to a single flash can be considerably delayed by interposing a second flash shortly before the deflection caused by the first is due to occur. How can these lengthenings of the latent period be explained?

Granit [1933] has produced evidence that of the three components of the retinal action currents the positive P II alone is associated with the discharge of impulses along the optic nerve, while the negative P III tends actively to inhibit the discharge. The inhibitory component P III increases with intensity of stimulation. Our records show that it is often well developed in the response to short flashes, where it manifests itself both as an α -wave and, in one experiment, by the more complex effect illustrated in Plate I, fig. 1. It seems probable therefore that in repetitive stimulation P III inhibits and counteracts for a time the rise of P II which would otherwise have occurred in response to the preceding flash.

P I is left out of account for reasons which have been given above. It appears to play no rôle in the production either of oscillations in the curve of retinal potential or [Granit, 1933] in the setting up of impulses in the optic nerve. The conclusion that the negative component P III has a share in the reaction to intermittent light was also reached by Piper [1911] and is in keeping with Renqvist's observation [1924] that flashes superimposed upon steady illumination give rise to both α - and *b*-waves in the frog's retina. Our own evidence, with an eye in which the negative component is small, is less direct.

With weak stimuli and a steady slow rate of intermission, the ripples may be larger and rise more steeply than the initial *b*-wave for a continuous stimulus of the same intensity. This can best be accounted for on the

basis of interaction or mutual facilitation between the excitatory effects of the individual flashes.

Spatial summation is shown by the influence of area both on the latent period for steady illumination and on the fusion frequency for intermittent stimulation. Areal effects are also perhaps illustrated in another way. Measurement of the records in three long series of experiments with intermittent stimuli shows that a given change of area always has less effect than a corresponding change of intensity on the latent period of the ripples (see Table II). (It should perhaps be mentioned that in one of these the ratio D/L fell steadily from 54 to 45 p.c. as the area was decreased a hundredfold and in Table II it falls with decreasing brightness, but these are exceptional.) Now the latent periods of the ripples are, of course, all longer than those of the initial b -wave of a continuous stimulus would be. But since for a continuous stimulus the effects of change of area and change of intensity are much alike, and may be identical (as in Text-fig. 1 A), it would appear that the lengthening of the latent period resulting from repetitive stimulation increases with the area more than it does with the intensity. In other words, the interaction between the effects of successive stimuli which causes the latent period for repeated flashes to be longer than that for single flashes is more marked the larger the retinal image and the larger the number of active retinal neurones. This conclusion is no more than tentative. It could only be definitely established by making serial observations on the effects both of intermittent and of steady stimuli on one and the same preparation.

A complete explanation of all our findings in terms of a complex process of interaction between excitatory and inhibitory components would admittedly be speculative. In the case of the latent period, however, it appears not only plausible, but also highly probable. But to go further and formulate a full-fledged theory of fusion frequency would now be premature. If fusion be due, for example, to two processes of opposite sign just balancing one another, the time relations of the rise and fall of both P II and P III would have to be considered. The data on these points for short flashes are at present unprocurable. There is the further difficulty that the initial b -wave is different from the intermittent " b -waves" evoked by the later flashes.

At high intensities and a steady rate of alternation, the first few flashes cause no rippling of the large initial b -wave, and the earlier ripples are smaller than the later ones. Among several possible factors at work in this, the three following are worthy of mention. Some form of inhibition may at first constitute a block. Or there may be some "inertia" to be

overcome before the semi-stationary state of balance between the processes co-operating to form the intermittent waves is reached. Or some response of an "all or nothing" character may at first be evoked. The last might take the form of a rapid utilization of some photochemical substance, which is only resynthesized after some time in sufficient quantity to be again capable of reacting. Whatever may be the true explanation, factors must obviously be taken into account which are unpredictable from a study of single flashes.

We have dealt rather fully with certain complicated effects. They must not, however, be allowed to distract attention from our main result, viz. that the ripples of intermittent stimulation are chiefly due to the rise and fall of P II during the bright and dark intervals. Piper [1911], at a time when much less was known about the nature of retinal action currents, came to essentially the same conclusion for eyes in which, as in the cat, the negative initial *a*-wave and the off-effect are small. For eyes in which they are large, he concluded, on the other hand, that the reaction to each flash was a negative *a*-wave, and to each dark interval a positive off-effect in which a small *b*-wave might be merged. His evidence for this view is partly derived from the order of magnitude of the latent period and partly from the responses to single flashes and to momentary interruption of a continuous stimulus. Such arguments do not appear conclusive. We have shown that the latent period of the intermittent waves in the cat's eye is identical neither with that of the *b*-wave for a single flash nor with that of the *a*-wave. Moreover, it seems unlikely that the retinae of different vertebrates would differ fundamentally in their response to intermittent light.

Doubtless, when off-effects are large, they may contribute to the oscillations, thereby illustrating the interaction between P II and P III on which we have already laid emphasis. But the response to intermittent stimulation cannot, we believe, be immediately reconstructed from data provided by a study of single flashes and brief interruptions of a steady light.

With stimuli which are not intermittent, the most striking of our findings has been that whereas area and intensity have pronounced effects on the length of the latent period, a flash lasting 4σ gives a response after precisely the same interval as does a similar stimulus of indefinite duration. This can only be interpreted as evidence that the latent period is determined much less by the velocity of the initial photochemical reaction than by nervous interaction at more proximal levels in the retina. In contradistinction to this, the amount of potential developed is often less for a short than for a long flash.

Sensory flicker and fusion.

Hitherto we have deliberately avoided making more than the barest of reference to the sensory phenomena of flicker and fusion. The experimental enquiry has been devoted entirely to retinal processes. It remains to indicate briefly the obviously close analogies which these bear to the sensations which they may ultimately evoke. For technical reasons the fusion point as determined from records of retinal action potential may well be lower than that which is perceived. The retino-cerebral apparatus which forms the physical basis of events in consciousness is probably more sensitive, and more stable, as a recording mechanism than the amplifier and string galvanometer with which our results have been obtained. Nevertheless, the two criteria may be expected to resemble one another in broad outline. This is found to be the case [cf. Sachs, 1929].

Fusion frequency, expressed in flashes per second, is roughly proportional to log intensity and, within limits, to log area, no matter whether the criterion be sensory [Ferry-Porter law; Granit and Harper, 1930], or the retinal action potential (*supra*), or the impulses in the optic nerve [Adrian and Matthews, 1928].

For rod vision the fusion frequency is lower than for cone vision [Schaternikoff, 1902; Lythgoe and Tansley, 1929; Creed and Ruch, 1932]. The same is true of retinal action currents [Piper, 1911].

SUMMARY.

1. The action potential of the dark-adapted retina of the decerebrate cat has been recorded from the eye *in situ* with the aid of valve amplification and a string galvanometer.

2. The area and the intensity of the illumination of the object used as stimulus affect both the latent period preceding the development of action potential and the height and steepness of the *b*-wave.

3. The latent period for the shortest flashes (4σ) is the same as that for prolonged stimulation, although the amount of potential which is developed may be less. Shorter flashes could not be obtained with our apparatus. It is probable that with them some lengthening of the latent period would be found, but the effect cannot be comparable with that of diminished area or intensity.

4. With intermittent stimulation at slow rates, there are ripples in the curve of retinal action potential. If a large *b*-wave is present, the rhythm of the stimulus may not appear in the tracing until several flashes have been delivered. Thereafter the amplitude of the oscillations and their

latent period remain constant during a period of at least 5 sec., but the latent period is usually much longer than that following the commencement of steady stimulation. With weak stimuli the ripples may be larger and may rise more steeply than the initial *b*-wave of an otherwise similar steady stimulus.

5. Above a certain frequency of alternation, the ripples disappear. Control experiments show that the "fusion frequency" is not affected by the properties of the recording apparatus.

6. The fusion frequency varies directly as log brightness and log area of the stimulus over at least a hundredfold range. Variations in brightness usually have more effect than variations in area. The product of fusion frequency and latent period of ripples is always found to be approximately constant. The duration of the flash at the fusion point is about 44 p.c. of this latent period.

7. The retinal process chiefly responsible for the ripples is the component P II [Granit, 1933] of the retinal action potential. P III probably interacts with P II and may, in so doing, affect the fusion frequency.

8. With rapidly intermittent stimulation, the smooth curve of action potential approximates closely to that given by a steady stimulus of half the intensity. The initial *b*-wave of the former, however, often occurs after a longer latent period than that of the latter. This is especially true with intense stimuli.

9. A flash delivered shortly before the end of the normal latent period following a preceding flash may greatly delay the expected movement of the string. Once the delay is over, the effect of the second flash summates with that of the first.

10. In the discussion, the various lengthenings of the latent period which have been found in repetitive stimulation are attributed to the inhibitory influence of the negative component P III on the positive component P II. Evidence of temporal and areal summation of effects is also presented.

11. Piper's views are briefly discussed, and the impossibility of predicting the results of intermittent stimulation from a study of single flashes and momentary interruption of steady illumination is emphasized.

12. The resemblance is pointed out between findings obtained by the study of retinal action potentials and the sensory phenomena of flicker and fusion.

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SIZE CHANGES IN THE SEMINAL VESICLES OF
THE MOUSE DURING DEVELOPMENT
AND AFTER CASTRATION.

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I. INTRODUCTION.

THE activity of testis hormone preparations might theoretically be demonstrated by reversing any of the various changes which follow castration, but the most hopeful results have naturally been obtained by restoring the structure or function of the accessory reproductive organs.

The sensitivity of the various tests which have been used is very different, but most of them appear to be satisfactory from a qualitative point of view. On the other hand, considerable difficulty has been experienced in developing methods of quantitative assay on mammals. The cytological appearance of the seminal vesicles, prostate and Cowper's glands of the rat and guinea-pig, and the motility of spermatozoa after castration, have all been used by the Chicago workers as qualitative or roughly quantitative indicators of activity, but for purposes of accurate assay they prefer the capon comb [Moore, Hughes and Gallagher, 1930; Moore, Price and Gallagher, 1930; Moore and Gallagher, 1930; Moore and Koch, 1932]. Voss and Loewe [1930, 1931], noting that cytological normality of the epithelium of the seminal vesicles was restored before macroscopic changes in the organ became appreciable, abandoned the attempt to use the gross size changes in favour of measurement of the height and mitotic activity of the vesicular epithelium. Martins and Rocha [1930], also working with mouse seminal vesicles as test objects, found them very variable in 16-21 g. mice, and used a length \times breadth index to express their gross size. Laqueur and the Amsterdam school [Freud, de Fremery and Laqueur, 1932] and Schoeller and Gehrke [1931] appear to rely mainly on the capon

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comb technique. Korenchevsky [1932] writes: "An average increase of 40 p.c. in the weight of the prostate with seminal vesicles of the injected pair, as compared with the control pair, was suggested above as a quite definite proof of the activity of testicular extracts and perhaps as a basic figure for one rat unit."

Activity of a preparation may be demonstrated in two ways: (a) the atrophy or non-development of an organ or a function after castration may be prevented by administration of the hormone, or (b) atrophy already effected may be abolished by injection. The application of either principle to assay work is, however, complicated by the difficulty of assessing the physiological or morphological reactions in quantitative terms. This difficulty is due to the lack of a definite end-point in most of the reactions and to the great individual variation which is found both in the normal and atrophied organs and in capacity to respond to the hormone. Further, the gross size of the seminal vesicles or prostate, which could be most easily assessed quantitatively, appears to be the least responsive to the hormone. A practical disadvantage is that most of the tests involve killing the animals, which can therefore be used only once.

It is preferable that routine assay should be as simple and as rapid as possible, and it is clear that some method based on the gross size of the seminal vesicles would be highly desirable. Such a method is likely to require longer administration of the hormone than is necessary with more sensitive indicators, but in view of its increasing availability the larger amount of hormone required is not a serious drawback, and for laboratory routine the elimination of histological examination is a great convenience. As a further practical point it seemed desirable to devise a method in which the use of adequate numbers of animals would avoid the necessity of collecting litter mate controls at every test. It seemed essential, however, that any system of assay depending on the development, maintenance or restoration of the gross size of the seminal vesicles should be based both on a detailed investigation of the size of these organs in the normal male, and on the relation between the initial weight and the ultimate size after castration. The present paper records the results of this preliminary work.

II. TECHNIQUE.

Mice were used from the albino colony previously maintained at University College, and now transferred to the National Institute for Medical Research. These have been colony-inbred for 11 years. Castration was performed in the ordinary way through the scrotum. At autopsy,

the animals were killed with chloroform, and the pelvic organs fixed whole in alcoholic Bouin's fluid. Preliminary fixation avoids the necessity of dissecting out the whole reproductive tract to prevent the loss of secretion which otherwise occurs when the glands or their ducts are cut in the fresh condition. After overnight fixation the glands were transferred to 70 p.c. alcohol, dissected out and weighed. The seminal vesicles of the mouse are

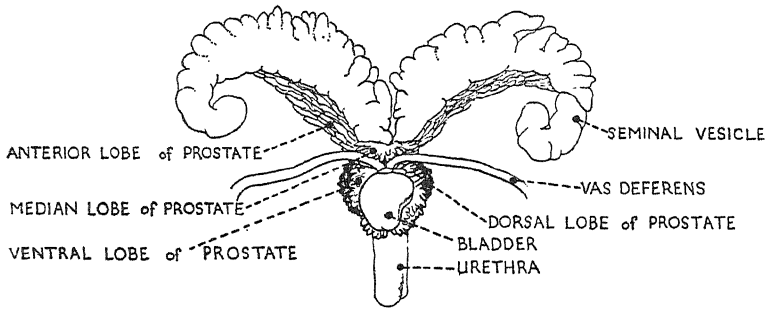


Fig. 1 A.

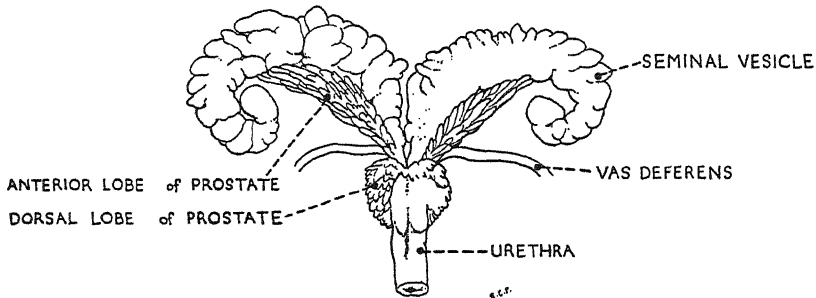


Fig. 1 B.

Fig. 1. A, ventral view of seminal vesicles, prostate and bladder of mouse; B, dorsal view of same region.

comparatively free in the abdominal cavity, being anchored only where they enter the urethra. The prostate (Fig. 1 A and B) consists of paired anterior lobes and a small inconspicuous median lobe attached to the base of the seminal vesicles, together with paired ventral lobes behind the neck of the bladder and paired dorsal lobes. The gland differs from that of the rat in the larger proportionate size of the anterior lobes, which in the mouse constitute about two-thirds of the total prostate. In the rat and guinea-pig the anterior lobes are said to differ histologically and function-

ally from the rest of the prostate, and to regress rather less rapidly after castration [see Moore, Price and Gallagher, 1930]. Complete separation of the prostate requires careful dissection even in the rat [Korenchevsky, 1932]; in the mouse it is difficult. When the glands are fixed *in situ*, however, there is little to be gained by taking the whole prostate. As a rapid and easily reproducible dissection we have therefore made a clean cut just in front of the bladder at the level of the insertion of the vasa deferentia, thus removing the whole of the seminal vesicles, and also the anterior lobes of the prostate. When the glands are fully grown, the prostate comprises up to 20–30 p.c. of the weight of the total prostate-vesicle complex. Since our technique removes only the anterior lobes of the prostate, only 15–20 p.c. of the removed tissue is prostate where the glands are fully grown. For brevity in this paper, the term “seminal vesicles” is used to denote all the removed tissue. Owing to the more fibrous nature of the prostate, its size atrophy after castration is proportionately less than that of the seminal vesicles, so that the atrophied glands include a rather larger percentage of prostate.

Statistical methods. The errors given are standard errors which, together with the coefficients of correlation, regression formulæ, etc., have all been calculated in the ordinary way. The abbreviation s.v.w. is used throughout for “weight of the seminal vesicles.”

III. S.V.W. IN RELATION TO BODY WEIGHT.

Fig. 2 shows s.v.w. plotted against body weight in 104 mice ranging from 14 to 32 g. A glance at the distribution of these points shows that the size increase has three phases. By 19 g. body weight the glands are very variable and often still insignificant; a period of rapid development then sets in, and by 24 g. body weight something approaching full size is attained. There is only a slight rise in s.v.w. as the body weight increases above 24 g. In view of these definite indications, the data were treated by calculating regression formulæ for the s.v.w. at the body-weight groups, 14–19 g., 19–24 g., 24–32 g. The results are given in Table I, which shows

TABLE I.

Body weight of group (g.)	Mean weight of seminal vesicles (mg.)	Coefficient of variation $\left(\frac{\sigma}{\text{mean}} \times 100\right)$	Coefficient of correlation	Regression formulæ for weight of seminal vesicles mg. (<i>y</i>) in terms of body weight g. (<i>x</i>)
14–19	42.5	66.3	0.454	$y = 8.98x - 114.9$
19–24	122.7	55.5	0.749	$y = 31.88x - 543.9$
24–32	246.3	30.5	0.219	$y = 7.99x + 32.5$

for each body-weight group, the mean s.v.w., the variability expressed by the standard deviation as percentage of mean, the weight of the seminal vesicles mg. (y) in terms of body weight g. (x), and the coefficient of correlation between the two.

The practical conclusion to be drawn from these results is that in mice of 24 g. and over, variation in s.v.w., though still considerable, is less than in smaller mice, and the average increase as body weight becomes greater is comparatively slight; the average s.v.w. considered as p.c. body weight is roughly constant. It follows that mice of 24 g. body weight or over are

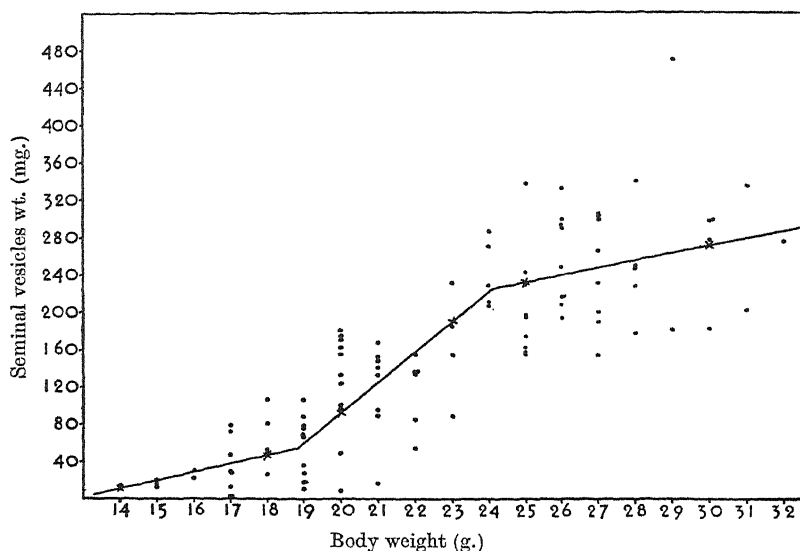


Fig. 2. Weight of the seminal vesicles of the mouse plotted against body weight.

the most homogeneous adults for experimental work involving seminal vesicle size. We have not ascertained to what extent, if any, the s.v.w. in mice above 24 g. is correlated with age or sexual activity, but we have observed a tendency for the vesicles of old mice to be small for their body weight. All of the mice dealt with here had been isolated from females.

IV. S.V.W. AFTER PRE-PUBERTAL CASTRATION.

Fig. 2 indicates that there is no appreciable development of the seminal vesicles below 14 g., corresponding to an age of 4-5 weeks. Twenty mice castrated below this age and weight were killed 8-12 months later. The s.v.w., in so far as it could be determined, varied between 1 and 3 mg.

With such small organs the actual arithmetic mean of our figures (1.8 mg.) serves only as an indication, and for practical purposes the s.v.w. after pre-pubertal castration can be taken as 2 mg. The use of the undeveloped seminal vesicles as test objects will be discussed in a later paper.

V. S.V.W. AFTER POST-PUBERTAL CASTRATION.

The atrophy of the seminal vesicles after post-pubertal castration was studied in 182 mice, varying in body weight from 20 to 33 g. at the time of castration. They were killed in groups of approximately 20 at times ranging up to 15 weeks' post-castration. Most of the mice were over 24 g. at the time of castration, but two groups of mice of 20–23 g. were included for comparison. The data are given in Table II, in which the

TABLE II. Post-castration atrophy of the seminal vesicles.

Days after castra- tion	Group 24–33 g. body weight			Group 20–23 g. body weight		
	No. of mice	Average body wt. g.	Average seminal vesicle wt. mg.	No. of mice	Average body wt. g.	Average seminal vesicle wt. mg.
0	45	27	246	29	21	126
7	21	28	129	—	—	—
14	20	25	70	—	—	—
21	21	25	44	—	—	—
24	—	—	—	15	21	20
35	23	26	34	—	—	—
52	22	26	22	—	—	—
70	19	27	26	—	—	—
78	—	—	—	22	21	10
105	19	27	18	—	—	—

two groups "0 days" after castration represent normal material taken from Fig. 1. In the 24–33 g. body-weight group it will be seen that the average weight for normal seminal vesicles sinks very rapidly after castration, about one-half of the weight being lost by the end of the first week. The decrease in weight seems to be caused chiefly by the loss of secretion from the seminal vesicles which accompanies the cytological degeneration described by Moore, Hughes and Gallagher [1930]. These workers find that almost the maximum amount of degeneration of the rat seminal vesicles is attained 10 days after castration. In the mouse atrophy is far advanced at 3 weeks after castration, but a further slight decrease takes place up to 7 weeks. Beyond this time there seems to be no further significant size change in the seminal vesicles. The top line in Fig. 3 shows the curve given by these results.

The 20–23 g. body-weight group shows a similar state of affairs, so far

as we have determined the curve. The seminal vesicles start at almost exactly half the size of those of the heavier mice. At 24 days after castration they weigh rather less than one-half those of the heavier mice, and at 78 days much the same relation is found (Fig. 3, bottom line). The weight of the glands at any given time after castration would thus appear to be roughly proportional to their original weight.

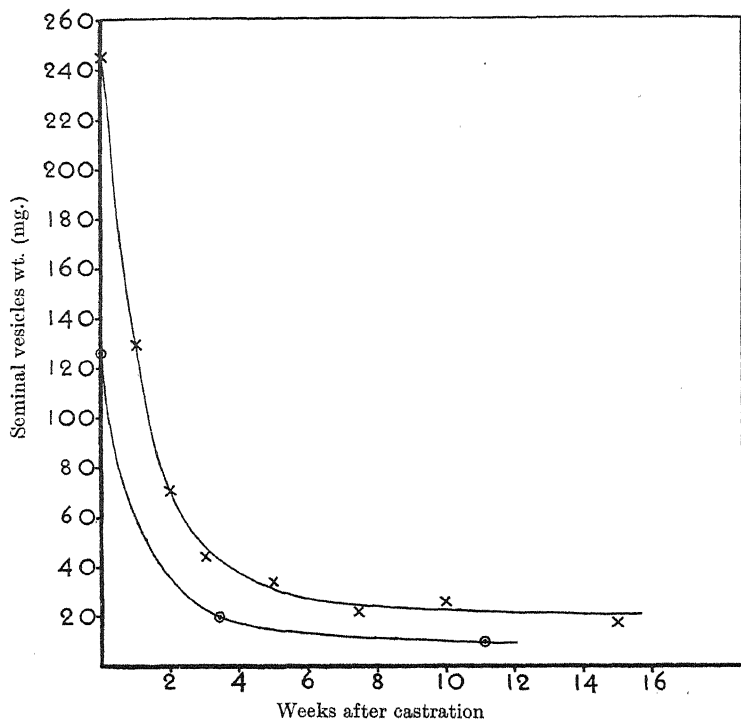


Fig. 3. Atrophy of the seminal vesicles after post-pubertal castration.

This relationship could be analysed in another way. We have shown (Table I) that there is a correlation in normal mice between body weight and s.v.w. A correlation between body weight at castration and the s.v.w. at any given time afterwards would almost certainly indicate a correlation of atrophied s.v.w. with original s.v.w. One would expect to be able to demonstrate this most easily on the smaller mice with the high correlation between body weight and s.v.w., but since we have concentrated on the heavier mice our material is inadequate to deal with this aspect of the question.

VI. DISCUSSION.

The results described above show that, in spite of considerable variability, the atrophy of the seminal vesicles of the mouse after post-pubertal castration appears to be comparatively regular when studied on groups of about 20 mice, and it may therefore be used as a basis of quantitative assay of the testis hormone. There can be little doubt that the heavier mice offer the best possibilities for this work. As shown in Fig. 3, the percentage decrease in size of the small seminal vesicles of the 20-23 g. mice is as great as that occurring in the larger ones of the 24-33 g. animals, but two facts make the latter preferable, (a) the much smaller initial variation in the weight of the glands and its low correlation with body weight (see Table I), and (b) the greater accuracy of preparing and weighing the larger glands.

For the 45 normal mice of the 24-33 g. group the mean s.v.w. is 246 ± 11 mg. At 7 days after castration the mean s.v.w. in 21 mice of the same weight group is 128 ± 16 mg. The difference, 118 ± 19 mg., is highly significant. The mean s.v.w. for the groups of mice at later stages after castration have errors of about the same order, and the greater differences of the means from the pre-castration size are correspondingly more significant. At 15 weeks after castration the mean s.v.w. is 18 ± 2 mg., the difference from normal being 228 ± 11 mg. Allowing for a similar order of variability and for using groups of mice of about the same size, statistically significant results could be obtained by the elimination of (1) 33 p.c. of the atrophy at 7 days after castration, or (2) 20 p.c. of the atrophy at 15 weeks after castration; such results could be used as a basis of testis hormone assay. The first would involve starting injections immediately after castration, with the aim of preventing the usual degree of atrophy; the second would constitute the partial or entire restoration of the already atrophied glands. The relative merits of these two procedures from the point of view of assay will be dealt with in a later paper.

VII. SUMMARY.

1. The normal size increase and post-castration atrophy of the seminal vesicles and attached anterior lobes of the prostate have been studied in 286 albino mice.

2. At 14 g. body weight the seminal vesicles are still minute, and in mice castrated below this weight the s.v.w. is only about 2 mg.

3. The seminal vesicles of the mouse undergo their main enlargement

at 19-24 g. body weight ; over this range their size shows a high correlation (0.749) with body weight. In mice above 24 g. the glands are less variable and show a low correlation with body weight (Table I and Fig. 2).

4. The average weight of the seminal vesicles (including the anterior lobes of the prostate) from 45 mice of 24-33 g. body weight was 246 mg., that of the glands of 29 mice of 20-23 g. body weight was 126 mg.

5. After post-pubertal castration, the glands lose about half their weight within 7 days, and then gradually sink to a nearly constant level of less than 10 p.c. of their original weight (Fig. 3).

6. There is a reasonable hope that the gross size of the mouse seminal vesicles may be used as a basis for assaying the testis hormone, provided that adequate numbers are used to allow for the considerable variation.

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THE EFFECT OF THE INTRAVENOUS ADMINISTRATION OF WATER UPON THE RATE OF URINE FORMATION.

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It is of importance to know whether absorption through the alimentary mucous membrane is an essential preliminary to water diuresis, for some workers have reported that no diuresis occurs when water is injected intravenously. Cow [1914] suggested the presence of a diuretic hormone in the intestinal mucosa, and other workers have thought that such a hormone might be present in the liver.

When water is given orally diuresis begins after about 20–60 min., and it is of interest to know how much of this delay is of enteral origin.

EXPERIMENTAL EVIDENCE.

A. *Rabbits.*

About 1 p.c. of the body weight of tap water at 37° C. was injected into the marginal vein of an ear, locally anæsthetized with 2 p.c. novocaine. No general anæsthetic was given at this stage in any of the experiments. Female animals were used, and the bladder emptied periodically by expression. The rabbits sat quietly in a box during the injection and were free for the rest of the experiment. In all experiments the rabbits had been kept for 24 hours on a diet rich in cabbage which, as is well known, increases their susceptibility to water diuresis. Animals fed on oats, even with unlimited water, have much smaller urinary outputs.

(1) *Hæmoglobinuria.*

Hæmoglobinuria was usually observed. It was very variable in degree, usually of short duration and bore no apparent relation to the delay in onset, the degree or duration of diuresis. The delay in onset was certainly

present in a few animals where there was only a trace of hæmoglobin present in the first urine sample obtained after injection (see also the findings in cats).

When about 2 c.c. of a hæmoglobin solution, prepared by alternate freezing and thawing of the animals' own blood, were reinjected intravenously, no change in the rate of urine formation was observed in three out of three cases in spite of the occurrence of hæmoglobinuria. The urine was collected for 2.5 hours after the injection.

It appears from these observations that the presence of free hæmoglobin in the blood does not of itself cause diuresis.

(2) *The effect of intravenous water on the rate of urine formation.*

In eighteen out of twenty-two rabbits a copious diuresis followed the intravenous injection of water. In six cases the diuresis was allowed to proceed without interruption. Five of these animals excreted 140–250 p.c. of the volume of water injected and one excreted 110 p.c. At the peak of diuresis the rate of excretion was from ten to fifteen times the original rate, the urine was paler in colour and the urea percentage had fallen considerably in all cases where it was estimated.

(3) *Comparison between the time relations in diuresis, from water given intravenously and enterally.*

In animals receiving injections of water intravenously, diuresis started after an average interval of 60 min.; 15 min. was the least interval (exceptional), and 105 min. the longest.

When 4 p.c. of the body weight of water is given by stomach tube to rabbits, the diuresis also starts about an hour after giving water. When small doses of water (say 1 p.c. of the body weight) such as were injected intravenously are given by stomach tube, very variable results are obtained owing no doubt to the large amount of food residue contained in the stomach and intestines.

Nevertheless, it seems probable that the delays in onset are not very different because in man and in rats (unpublished observations) the delay after drinking water is not greatly altered by increasing the dose of water administered.

(4) *Factors inhibiting the diuresis produced by intravenous water administration.*

(a) *Anæsthetics.* In four experiments the animals were lightly anæsthetized with ether and in five experiments with chloroform. In most cases the anæsthetic was given as soon as the urinary output had increased

threefold or more. It will be seen from section (2) that in general a still greater rate of urine formation was to have been expected, on measurement of the next urine sample, had no anæsthetic been given. In all of these experiments the anæsthetic caused a marked inhibition of urine formation (Fig. 1).

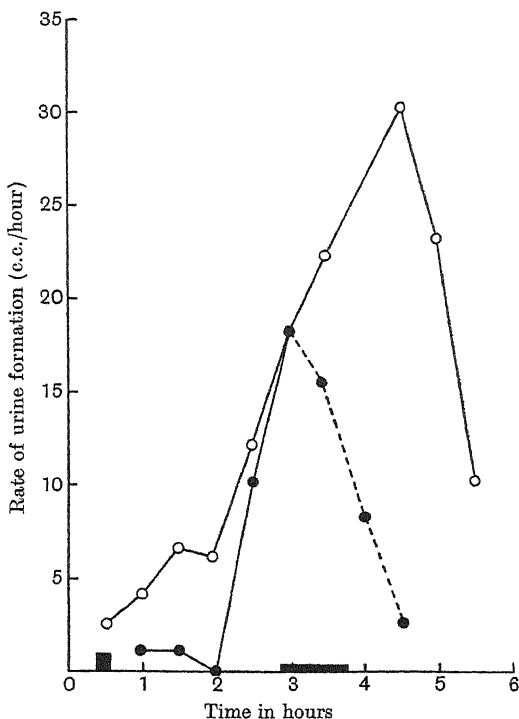


Fig. 1. The effect of ether on the diuresis after intravenous water. ○ Diuresis uninterrupted. ● --- ● Diuresis interrupted by ether. ■ 25 c.c. water given intravenously. ■ Ether administration. The same rabbit was used for both experiments.

In all of three experiments where the rate of urine formation was followed for some time after recovery from the anæsthetic (Fig. 2) a renewal of the diuresis was observed, which took place when the animals had excreted all of, or more than, the additional water given. In one case the second diuresis was large and the amount of urine much greater (170 p.c.) than the amount of water given. It seems that the mechanism causing diuresis is not directly controlled by the excess of water then present in the body as a whole, since the diuresis is renewed at a time when there is

no excess of water. This is consistent with the experiments of Heller and Smirk [1932 *a, b*], where diuresis was obtained in rabbits and rats, previously depleted of as much water as was subsequently given.

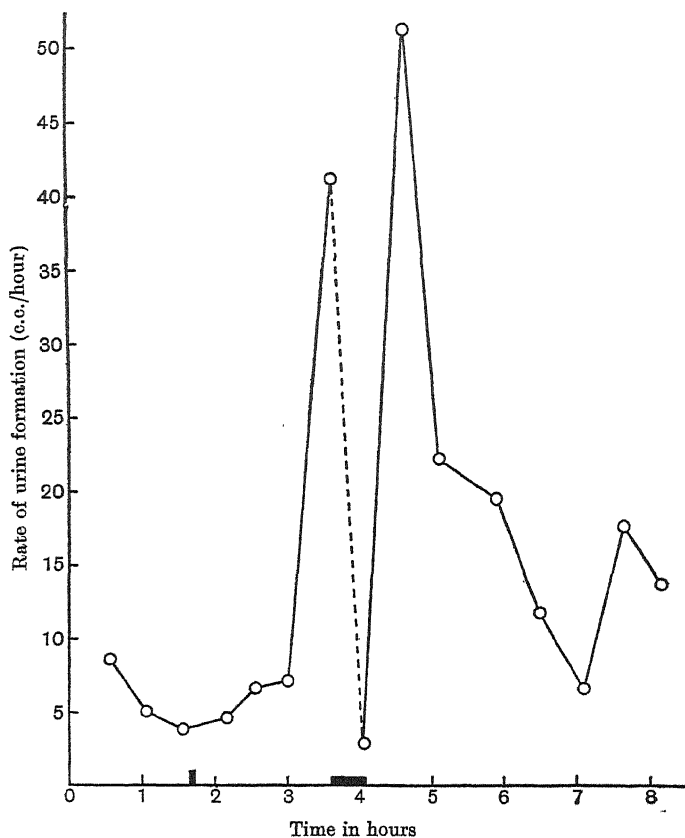


Fig. 2. The effect of chloroform on the diuresis after intravenous water. ○ --- ○ Diuresis interrupted by chloroform. ■ 25 c.c. water given intravenously. ■ Chloroform administration.

(*b*) *Struggling*. It is well known that violent exercise inhibits water diuresis. On this account a few experiments were performed to see if the results described in the previous section might be partly or wholly explained by a struggling of the animals during the induction of anæsthesia.

The rabbits were, therefore, held with their noses in the anæsthetic mask and struggling induced by filling the mask with tobacco smoke. In

two of our three experiments the rate of urine formation fell temporarily below the previous rate. This represents a greater inhibition than the actual measurements suggest owing to the fact that the urine output was on the increase. The degree of inhibition is much less, however, than that observed with anæsthetics which reduce the output to the basal rate or even below.

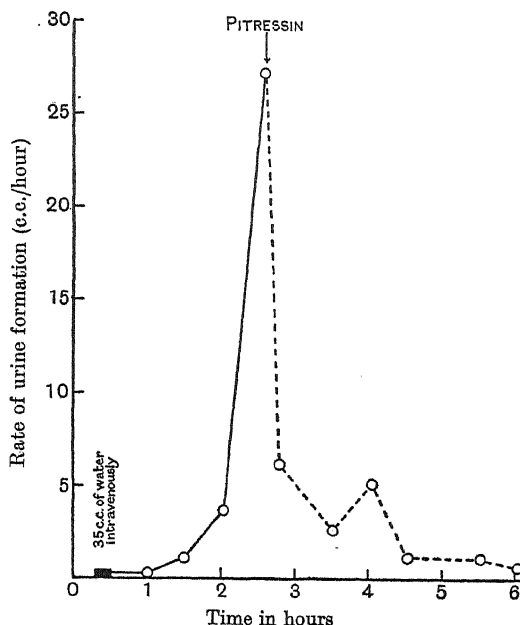


Fig. 3. The effect of pitressin on the diuresis after intravenous water. This experiment was performed on the same rabbit as those illustrated in Fig. 1.

(c) *Pituitary hormone (pitressin)*. In all of five experiments the diuresis after intravenous water was inhibited by the injection of 0.05–0.1 c.c. of pitressin, administered either intravenously or subcutaneously (Fig. 3).

B. Cats.

Three groups of experiments were performed on cats. In each case the animal was anæsthetized with chloroform, and a cannula inserted into the saphenous vein. The chloroform was discontinued, and immediately 0.05 g. per kg. (except where otherwise stated) of body weight of chloralose, in 2 p.c. solution of normal saline, was run into the vein. Through a suprapubic incision, a cannula was inserted into the urethra so that it just entered the bladder. Finally, both the wounds were well swabbed with

percaïn (1/1000), and the suprapubic incision sewn up. Under these conditions the animal remained quiet for about 6 hours, at the end of which time it was killed with chloroform. For 2 or 3 hours after the urethral cannula was put in, the rate of urinary secretion was observed by quarter- or half-hourly readings of the urine volume. About 1 p.c. of the animal's body weight of glass-distilled water at 37° C. was then allowed to run into the saphenous vein. (There appeared to be no difference in the actions of distilled and tap water.) The rate of the injection varied, but averaged about 4 c.c. per minute. In the last few experiments made it was observed that if the tubing and cannula (capacity 2 c.c.) connecting the water burette to the vein were filled with normal saline, the incidence of hæmoglobinuria was much reduced.

(1) *The effect of intravenous water on the rate of urine formation.*

The results are summarized in Table I; in nine out of the twelve cats a diuresis followed a single injection of water. In two of the remaining

TABLE I. The effect of intravenous water on the secretion of urine in cats anesthetized with 0.05 g. of chloralose per kilogram of body weight.

Hæmoglobinuria: "Slight" \equiv urine clear within 1 hour						
+ \equiv urine clear in 2 hours						
+ + } \equiv urine coloured after 2 hours						
+ + + }						
Cat	Interval between in- jection and diuresis min.	Duration of diuresis hours	Max./initial rate of urine for- mation	Increase in output of urine, expressed as p.c. of H ₂ O given	Hæmo- globi- nuria	Remarks
10	15	1½	14	90	Slight	—
11	1st water injection	—	(2.5)	—	+	Slight increase in urine output
	2nd water injection	15	2	11.5	87	+
12	55	2½	14	115	Slight	—
13	15	4	9.5	112	+	—
14	55	3½	27	56	+ + +	Continuous infusion of 85 c.c. in 2½ hours
15	40	1½	3	45	+	—
16	0	3	5	107	Slight	—
17	—	—	—	—	Nil	—
18	70	5 +	9	140 +	+ +	Diuresis still in pro- gress when killed
19	25	3	4.5	80	+	—
20	20	3	15	155	Slight	—
21	1st water injection	—	—	—	Nil	—
	2nd water injection	15	2½ +	6	83 +	Nil
Average	30	3	11	97		

three animals a second dose of water was given after a suitable interval, and in both cases a diuresis was then obtained. The extra amount of urine secreted during the diuresis varied, but the average of the experiments gave 97 p.c. of the amount of water given. The rate of secretion at the peak of diuresis was often increased from ten to fifteen times the normal rate. Delay between the administration of water and the onset of diuresis averaged about half an hour, being shorter than the corresponding average delay in rabbits.

It was thought unnecessary to control the hæmoglobinuria as a possible factor in causing the diureses, for it was completely absent in four cases. In others it was very slight and transient, and in no case did its severity appear to bear any relation to the time of onset, the amount, or the duration of the diuresis.

(2) *The effect of larger doses of chloralose.*

The results obtained are summarized in Table II. The animals in this group were given 0.075–0.1 p.c. of body weight of chloralose intravenously instead of the routine dose of 0.05 p.c. of body weight which was finally adopted. Only one of the five animals in this group gave a diuresis after a single injection of water. Two of the remainder were tried with a second dose, and one gave a moderate diuresis.

TABLE II. The effect of intravenous water on the secretion of urine in cats anæsthetized with 0.075 g. or more of chloralose per kilogram of body weight.

Cat	Interval between in- jection and diuresis min.	Duration of diuresis hours	Max./initial rate of urine for- mation	Increase in output of urine, expressed as p.c. of H ₂ O given	Hæmoglo- binuria	Dose of chloralose per kilogram of body weight g.
2	15	4	13	200	++	0.075
3	1st water injection	—	—	—	Slight	0.1
	2nd water injection	—	—	—	+	
4	1st water injection	—	—	—	Nil	0.075
	2nd water injection	40	1½	11.5	Slight	
5	—	—	—	—	Slight	0.075
6	—	—	—	—	+	0.075

This comparative failure to obtain diuresis where more chloralose was given suggested a parallel to the action of ether or chloroform on the rabbit diuresis.

(3) *The effect of evisceration on cats.*

The same procedure was followed as in section B (1), except that the animals were eviscerated. This was done after cutting between ligatures and in the following order, the inferior mesenteric artery, the superior mesenteric artery, the coeliac axis, the portal vein, and finally the rectum and cesophagus. The abdominal wound was swabbed with percaïn and sewn up.

Out of seventeen experiments on eviscerated animals, a diuresis was only observed in five cases, but these seem worth recording as being definitely positive results (Table III). The diureses were similar in cha-

TABLE III. The effect of intravenous water on the secretion of urine in eviscerated cats anaesthetized with 0.05 g. of chloralose per kilogram of body weight.

Cat	Interval between in- jection and diuresis min.	Duration of diuresis hours	Max./initial rate of urine for- mation	Increase in output of urine, expressed as p.c. of H ₂ O given	Hæmoglo- binuria
A	40	2½	3	90	Nil
B	25	2½	5	50	+
C	25	1½	4	35	Nil
D	25	3	15.5	90	Nil
E	55	2	5	100	?

acter to those obtained from the intact animals, except that the maximum rate of secretion never rose so high, and the amount of urine output was less. Nevertheless, they prove that a diuresis may result from the intravenous administration of water and that the presence of the alimentary canal is not essential for establishing such a diuresis. This finally disposes of the theory that the alimentary route of absorption is necessary for the production of a diuresis after giving water.

In one case only the liver was in the circulation, the coeliac axis having been ligatured distal to the origin of the hepatic artery. In the remaining four cases the liver was out of the circulation as was shown by the intravenous injection of dyes before death, which were seen to colour all tissues except the liver. It seems very probable that the presence of the liver is also unnecessary for the production of a water diuresis.

DISCUSSION.

It has been shown that in unanaesthetized rabbits the intravenous injection of water in sufficient amount causes diuresis. It is necessary to decide, however, if there is justification for calling this a water diuresis.

The characteristics of a water diuresis are that there is a definite delay before diuresis begins; that the rate of secretion rises rapidly to a peak, where it may be 10 to 20 times the normal rate; that it is of short duration (in hours); that the amount of urine secreted during the diuresis is of the same order as the amount of water given; and that it is cut short by pituitary hormone and inhibited also by certain anæsthetics such as ether and chloroform. Our results show that these criteria were fulfilled in the rabbits, and, so far as tested, in the cats, with the possible exception of the amount of water put out. Although this is irregular in the cats it is of the order of the amount of water given, but in the rabbits it is in excess of that given.

Our results also show that diuresis is not evoked by the intravenous injection of laked blood in amounts sufficient to cause hæmoglobinuria, and the water diuresis did not appear to be influenced, either in respect of delay before the onset or of the amount of urine formed, by the moderate degrees of hæmoglobinuria which usually followed intravenous injection of water; although gross hæmoglobinuria appeared to prevent or reduce the diuresis.

We, therefore, find ourselves in agreement with Frey [1907] and differ from Ginsberg [1912] and Cow [1912, 1914]. The proof that a diuresis of the water diuresis type results from the intravenous injection of water removes the need for assuming absorption of a diuretic hormone from the intestinal mucosa.

The inhibition of the diuresis in rabbits by ether, chloroform and pituitrin indicates that these substances have a parenteral action and that the reduction in diuresis which they cause is not due merely to prevention of alimentary absorption. This confirms the experiments of Heller and Smirk [1932 *b*].

In cats the use of the standard dose of chloralose as an anæsthetic did not seem to interfere with diuresis, but it may be significant that in those cats which received more than the standard dose there was little success in the attempts to procure a diuresis (see Table II).

Our experiments have a further bearing on some previous work by Heller and Smirk in which rats, previously depleted of water, were given a dose of water by mouth insufficient, or just sufficient, to restore their normal water content. These animals had a diuresis, in which the amount of excess urine secreted was not much less than that secreted by controls, in which all the water given constituted an excess over their normal body content. Our present experiments discount the possibility that this diuresis might have been due to the stimulus of a temporary

excess of water in the gut, or to preferential storage of water in the liver.

The experiments on eviscerated cats were designed to see if a diuresis of the water diuresis type could be produced after complete removal of the stomach and intestines and when the liver was out of the circulation. The conditions of the experiments were extremely unfavourable, as it has been shown that even simple laparotomy may be sufficient to inhibit water diuresis [Heller and Smirk, 1933]. In addition the longer and deeper chloroform anæsthesia necessary for evisceration no doubt played a part in preventing frequent success in this experiment. Again, in the simple diuresis experiments the diuresis was only obtained at the first water injection in nine out of twelve cats, and in eviscerated cats the general condition is not maintained long enough to permit of two trials. Five experiments, however, yielded a definite diuresis after water injection. This could not be accounted for by the picking up of some diuretic substance from the alimentary canal, which was absent; nor from the liver, which was completely out of the circulation in four experiments as proved by the intravenous injection of dyes.

A further point of interest is the latent period between water administration and diuresis. This is always seen when the alimentary route of absorption is used, and is also apparent in our experiments (though not demonstrable in cats 11 and 16). This confirms the work of Heller and Smirk [1932 *a*], Klisiecki, Pickford, Rothschild and Verney [1933] and Smirk [1933] who found that alimentary absorption is in advance of diuresis. In rabbits the delay in the onset of diuresis is about the same whether they receive water intravenously or by the alimentary tract; but individual variations in both cases preclude an accurate statement of the relative time intervals.

It is evident, however, that the delay when water is taken in the normal way is due only in part to the time spent in alimentary absorption. Other time-consuming processes must intervene between the initial dilution of the blood and the final excretion by the kidney.

SUMMARY.

1. Unanæsthetized rabbits and chloralosed (0.05 g. per kg.) cats have a typical water diuresis after intravenous injection of water. The urine formed is pale and hypotonic; the extra amount secreted varies, but in cats is of the same order as the amount of water given, and in rabbits more.

2. Intravenous water may cause hæmoglobinuria, but reasons are advanced for excluding this as a factor in causing the diuresis.

3. In rabbits the diuresis after intravenous water is inhibited by pituitrin, ether and chloroform. It follows that the antidiuretic action of pituitrin, ether and chloroform is obtained parenterally.

4. A diuresis after intravenous water has been obtained in cats from which the alimentary canal has been removed and where the liver has been excluded from the circulation. The portal route of absorption is not essential, therefore, for the production of a water diuresis.

5. There is a considerable delay in the onset of diuresis even when the water is given intravenously. This suggests that the time expended in alimentary absorption is not alone responsible for the latent period when water is given orally.

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ON THE PRESENCE OF "NOVADRENINE"
IN SUPRARENAL EXTRACTS.

By U. S. v. EULER.

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Stockholm.)

RECENTLY Szent-Györgyi and his co-workers [Annau, St Huszák, Svirebely and Szent-Györgyi, 1932] reported in this *Journal* on observations revealing a discrepancy between colorimetical and physiological adrenaline tests in extracts from bovine suprarenal medulla, which they thought to be explained most adequately by the supposition of a new and more active form of adrenaline. For this substance the investigators have proposed the name "Novadrenine."

The assumption that the effects observed cannot be attributed to adrenaline is based chiefly on the following observations:

(1) The adrenaline content of the bovine suprarenal medulla was found colorimetrically to be 1-2 mg. per g., whether the physiological activity of the extracts was high or not.

(2) The physiological activity of highly active extracts from the suprarenal medulla was found to correspond to 15-30 mg. of adrenaline per g. of tissue, or 10-20 p.c. of the dry weight, which is considered impossible.

The striking results obtained by Szent-Györgyi and his co-workers made it desirable to study the question of "novadrenine" further. The following experiments were carried out in the course of an attempt to work out a convenient method for estimating adrenaline colorimetrically. The adrenaline content of the suprarenal medulla from oxen has been studied in a number of experiments, the conditions under which Szent-Györgyi and his co-workers were able to obtain their active substance having been followed closely. One of the experiments, all of which gave the same results, will be communicated in detail below.

EXPERIMENTAL.

Immediately after death the suprarenals from an ox (weight 710 kg.) were prepared and put in a glass vessel in a Dewar's flask with cold mixture ($-6^{\circ}\text{C}.$). The glands arrived at the laboratory about $\frac{3}{4}$ hour after the death of the animal. The preparation was then proceeded with immediately. One gland was frozen by means of CO_2 and cut into slices of about 0.2 mm. in thickness, after removal of the cortex. The slices of medulla, weighing 4.5 g., were kept frozen until they were suspended in 9 c.c. of 0.5 p.c. trichloroacetic acid, that is 2 c.c. for each gram of pulp, as done by Szent-Györgyi and his co-workers. After thorough mixing the extract was rapidly heated to $80^{\circ}\text{C}.$ and quickly cooled down again with the aid of iced water. The mixture was then filtered and kept in the ice-box (temperature $-5^{\circ}\text{C}.$). The filtered solution was slightly opaque, but after standing in the ice-box for about an hour it passed through the filter quite clear.

The second gland of the pair after arrival at the laboratory was kept at room temperature. The preparation of the first gland having been completed, the medulla of the second one was separated by means of a scalpel. 3.6 g. of the medulla were then ground up in a mortar with $N/10$ HCl and fine sand, following the procedure of Folín, Cannon and Denis [1912-13]. After a short boiling, the addition of sodium acetate and reboiling, the extract was cooled down and filtered, giving a clear filtrate.

The adrenaline contents of the extracts were then immediately estimated biologically and colorimetrically.

In order to determine the physiologically active adrenaline the blood-pressure method was employed. The determinations were made on cats under ether and on rabbits anæsthetized with urethane. The standard used was Supraren. synth. cryst. pur. (Höchst). Both kinds of test animals gave the same results. Part of the tracings from one experiment with suprarenal extracts on the blood-pressure of a rabbit under urethane is given in Fig. 1.

For comparison the following solutions were used:

A. Medullary extract according to Folín and his co-workers; 1 c.c. extract corresponds to 0.8 mg. of the medulla.

B. Medullary extract according to Szent-Györgyi and his co-workers; 1 c.c. extract corresponds to 0.6 mg. of the medulla.

Standard, 0.005 mg. adrenaline per c.c.

All extracts were made up with saline, which was adjusted to a slightly acid reaction to litmus by means of HCl .

From the biological determinations it was found that: 1 c.c. *A* corresponded in action to 0.0114 mg. adrenaline, 1 c.c. *B* corresponded in action to 0.0078 mg. adrenaline, if 2.5γ adrenaline = $0.22 A = 0.32 B$.

From these figures the adrenaline content of the medulla would be: in the gland prepared *ad A* 14.3 mg. adrenaline per g. fresh medulla, and in the gland prepared *ad B* 13.0 mg. adrenaline per g. of fresh medulla.

Apparently the two methods used give about the same yield of adrenaline. The figures obtained from the experiment communicated

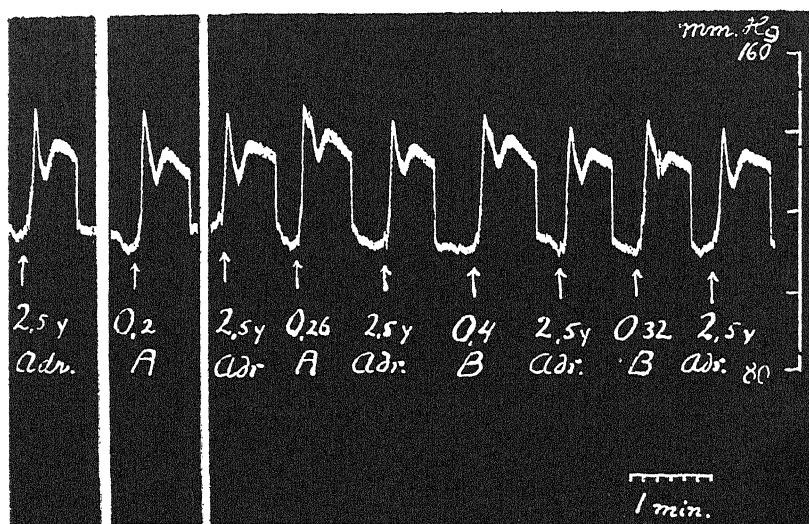


Fig. 1. Rabbit blood-pressure under urethane anaesthesia. 0.22 c.c. *A* (extract from bovine suprarenal medulla according to Folin) corresponds in action to 0.32 c.c. *B* (medullary extract according to Szent-Györgyi) and to 0.0025 mg. adrenaline.

thus agree fairly well with those found by Szent-Györgyi and his co-workers in active extracts, and also with those communicated in the recent review by Kojima, Nemoto, Saito, Sato and Suzuki [1932] with extracts from bovine suprarenal medulla.

The colorimetric determinations were carried out by a spectrophotometric method recently worked out which is based on the Vulpian [1856] reaction [Euler, 1933]. This method was found to give figures in fairly close agreement with those found biologically.

The instrument used was a Pulfrich "Stuphenphotometer" (Zeiss). The following figures were obtained by testing the extracts about 1 hour

after the commencement of the preparation. The extracts were 10 times as strong as those used in the rabbit blood-pressure experiment (extract *a* thus = 10 *A*, *b* = 10 *B*).

Extract	Adrenaline found colorimetrically
1 c.c. <i>a</i>	0.118 mg.
1 c.c. <i>b</i>	0.081 mg.

The amounts of adrenaline per g. of medulla will, from these determinations, amount to 14.7 mg. and 13.5 mg. per g. respectively.

The agreement between the two estimates, colorimetric and biological, hardly admits of the assumption of a special, more highly active adrenaline-like substance in this case, though the physiological activity of the extracts was of the same order as in the active extracts of Szent-Györgyi and his co-workers.

Further, the two methods of extraction gave practically the same amount of adrenaline as calculated per g. of medulla, though no precautions were taken as regards cooling etc. in the case of the Folin extract.

Other extracts were prepared after freezing the glands immediately after their arrival at the laboratory by means of liquid air. No significant difference could be detected, however, as to the adrenaline content as determined either colorimetrically or biologically.

It might thus be assumed that—as significant differences between the animals used in the experiments would hardly be the cause of the discrepancy—the colorimetric method used by Szent-Györgyi and his co-workers is less suitable. This suggestion is supported by the fact that the physiologically determined activities are of the same order in active extracts, though the colorimetric determinations differ.

The colorimetric method used by Szent-Györgyi and his co-workers is also based on the Vulpian reaction. The extracts were neutralized with a small excess of sodium bicarbonate. Even in slight alkaline solution, however, the colour is liable to fade away rather quickly, as I have had the opportunity of observing, so that, after a short time, the activity would appear less than the original. Szent-Györgyi and his co-workers note that the colour was stable a minute or so. It may be assumed, however, that the fading starts before the determination has been completed, thus giving unreliable results, unless the value at zero time has been calculated from the fading velocity in each case.

SUMMARY.

Extracts from the bovine suprarenal medulla have been prepared according to Szent-Györgyi and his co-workers, and their activity determined physiologically and colorimetrically.

No significant difference was observed between the activity as found by biological tests (rabbit blood-pressure) and that found colorimetrically (using the method of Euler).

An attempt to explain the different results is made¹.

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¹ This paper was kindly submitted by Dr U. S. v. Euler for my criticism. v. Euler is able to give an adequate explanation of the experiments of my collaborators and myself, without the necessity of supposing the existence of a more active form of adrenaline (novadrenine). I accept his view, by which the supposition of such a compound becomes superfluous.—A. Szent-Györgyi.

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577.153.2.033AN APPARATUS FOR THE PRODUCTION OF FINELY
DISPERSED EMULSIONS, AND THE RATE OF
DIGESTION OF FAT BY LIPASE IN RELA-
TION TO THE SURFACE AREA.

BY A. C. FRAZER AND V. G. WALSH.

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THE various types of apparatus available for the mechanical production of emulsions consist for the most part of plates or cones with small clearances between them, through which the materials are passed whilst the plates or cones are spun at very high speeds.

The particles in such emulsions are not uniform in size, nor are they very finely divided. Moreover, in order to obtain a permanent emulsion it is necessary to use high percentages of stabilizers. These emulsions tend to break during sterilization by heat, and are therefore unsuitable for injection purposes.

The apparatus described has been constructed on a different principle and depends primarily on a pulsating action by which the materials are subjected to alternate decreases and increases of pressure, and are then ejected at a very high velocity through a narrow gap. The materials are drawn into and intimately mixed in the cylinder by the suction action of a plunger, the aggregates are then disintegrated by the shearing force exerted during expulsion through the gap.

The operation of the apparatus can best be understood by reference to Fig. 1.

The cylinder *C* is provided with two inlets at the inlet head *A* and a single outlet *H* whilst a crank drive *G* reciprocates a plunger *B* inside the cylinder. The two phases of the ultimate emulsion are drawn through the two inlets into the cylinder by the suction stroke of the plunger *via* a non-return valve and the plunger then makes its compression stroke. When the pressure reaches a predetermined point in the cylinder the spring in the head *D* releases the outlet valve *E*, and the contents of the cylinder is

ejected through the narrow gap between the face of the valve and its seating.

The dispersion of the particles is effected by the internal friction during the expulsion of the materials, the greater the velocity the greater will be the internal friction and hence the smaller and more uniform will be the particles in the dispersed phase. Again the velocity is dependent on the size of the gap through which the materials have to pass. The size of the gap can be varied by altering the pressure exerted by the spring, by means of the hand-screw *F*. When the hand-screw is fully home the pressure in the cylinder necessary to lift the valve is 3000 lb./sq. in. and the gap is at a minimum.

The feeds are arranged by connecting two graduated glass containers to the two inlets by means of rubber tubing with suitable taps interposed. The two phases are placed separately in the containers, and by adjusting the rates of flow by means of the taps on the feeds any desired percentage of oil in the emulsion can be produced without mixing the materials beforehand. The operation of the apparatus is continuous and it runs at a speed of 275 cycles per minute, driven by a $\frac{1}{4}$ H.P. motor and the output is 2.1/hr.

The emulsions produced at high pressures are extremely finely divided and uniform. The particles show violent brownian movement and average about $\frac{1}{2}\mu$ in diameter. Although relatively small amounts of stabilizers are used, these emulsions are extremely stable and show no tendency to break when sterilized by prolonged boiling. Such high pressure emulsions are used by the authors for intravenous injections.

With these finely dispersed emulsions, investigations were carried out in order to determine the rate of digestion of olive oil by lipase in relation to the surface area of fat exposed to the action of the enzyme.

In the first series of experiments an emulsion of olive oil 20 p.c. in water was prepared by intermittent shaking, the continuous phase con-

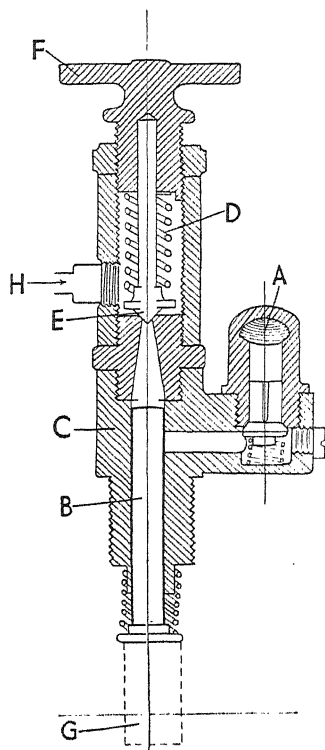


Fig. 1.

taining 5 p.c. of gum arabic. A similar 20 p.c. olive-oil emulsion was prepared by passing the oil and the continuous phase through the apparatus here described, using a pressure of 1000 lb./sq. in. on the valve. The globules in the shaken emulsion averaged $3-4\mu$ in diameter, whilst those of the emulsion prepared with the apparatus averaged $\frac{1}{2}-1\mu$.

Each emulsion was now rendered alkaline to phenolphthalein by addition of sodium carbonate, until a standard pink colour was obtained which was used as a control colour. The *pH* was 8.5, being the same in both emulsions.

40 c.c. of each emulsion was now taken and to each was added 10 c.c. of a lipase solution prepared by grinding 5 g. of pig's pancreas in 25 c.c. of water and after 30 min. straining it through muslin. The flasks were placed in the incubator at 37°C . At definite time intervals both flasks were titrated simultaneously with *N*/10 NaOH, the control colour being taken as the endpoint.

Results.

Incuba- tion time (min.)	c.c. <i>N</i> /10 NaOH	
	Coarse emulsion	Fine emulsion
15	7.8	21.3
75	28.3	68.2

This experiment was repeated, using the apparatus for the preparation of both emulsions. The coarse emulsion was prepared operating with a pressure of 200 lb./sq. in., and the fine emulsion with a pressure of 1000 lb./sq. in. The size of the globules in the case of the low pressure emulsion was from 2 to 3μ and the high pressure emulsion from $\frac{1}{2}$ to 1μ .

40 c.c. of each emulsion was taken and to each 10 c.c. of lipase solution was added. Both flasks were then incubated at 37°C . and titrated simultaneously with *N*/10 NaOH after definite time intervals as in the first experiment.

Results.

Incuba- tion time (min.)	c.c. <i>N</i> /10 NaOH	
	Coarse emulsion	Fine emulsion
15	11.0	25.9
75	36.9	56.0

In the second series of experiments only one titration with *N*/10 NaOH was performed in each flask. Twelve flasks were taken and numbered 1*A* to 6*A* and 1*B* to 6*B*. An emulsion of 20 p.c. olive oil in water, using 5 p.c. gum arabic as stabilizer, was prepared with the apparatus

using the high pressure. A similar 20 p.c. emulsion was prepared by intermittent shaking. Both emulsions were brought to a pH of 8.5, giving a pink colour with phenolphthalein, by adding a sufficient amount of sodium carbonate.

40 c.c. of the fine emulsion was placed in each of the flasks labelled *A* and 40 c.c. of the coarse emulsion in each of the flasks labelled *B*. To each flask was added one capsule of a commercial product "Holadin," which contains lipase and is supplied (B. and W.) in capsules each containing 3 grains of dried pancreas. The twelve flasks were then incubated at 37° C. After a certain time interval 1*A* and 1*B* were titrated simultaneously with $N/10$ NaOH until their respective control colours were reached. After a further time interval 2*A* and 2*B* were titrated, and so on until the six pairs of flasks had been titrated.

Results.

Incubation time (min.)	c.c. $N/10$ NaOH			
	Fine emulsion		Coarse emulsion	
15	1 <i>A</i>	26.2	1 <i>B</i>	4.4
30	2 <i>A</i>	36.5	2 <i>B</i>	9.9
50	3 <i>A</i>	41.1	3 <i>B</i>	13.2
80	4 <i>A</i>	46.8	4 <i>B</i>	17.6
120	5 <i>A</i>	52.6	5 <i>B</i>	26.3
150	6 <i>A</i>	56.3	6 <i>B</i>	31.8

Microscopical examination of the emulsions, at the time intervals taken, showed that coalescence of the fat globules and creaming was occurring in the fine emulsion.

This experiment was repeated, using emulsions of 10 p.c. olive oil in water. The same procedure was followed as in the previous experiment.

Results.

Incubation time (min.)	c.c. $N/10$ NaOH			
	Fine emulsion		Coarse emulsion	
15	1 <i>A</i>	12.2	1 <i>B</i>	3.9
30	2 <i>A</i>	17.2	2 <i>B</i>	6.3
50	3 <i>A</i>	22.6	3 <i>B</i>	9.0
80	4 <i>A</i>	26.0	4 <i>B</i>	11.7
120	5 <i>A</i>	28.7	5 <i>B</i>	17.4
150	6 <i>A</i>	30.1	6 <i>B</i>	19.9

DISCUSSION.

From the first series of experiments, it is apparent that the greater the surface area of fat exposed, the more rapid the rate of digestion by lipase. Thus the difference is greater between the rates of digestion in the fine

and coarse emulsions in the first experiment, in which the coarse emulsion was prepared by shaking, than in the second experiment, in which the coarse emulsion was prepared by passing the mixture through the apparatus under a pressure of 200 lb./sq. in. In both experiments the fine emulsions were prepared with the apparatus at a pressure of 1000 lb./sq. in.

From the figures obtained in the second series of experiments, curves were plotted (Figs. 2 and 3), the ordinates being the number of c.c. of $N/10$ NaOH required for each flask, the abscissæ the time intervals in minutes.

In Fig. 2, curve *A* was obtained from the hydrolysis of emulsion 1, prepared with the apparatus at a pressure of 1000 lb./sq. in., and curve *B* from emulsion 2, prepared by intermittent shaking. The diameter of the globules averaged $\frac{1}{2}\mu$ in emulsion 1 and 3μ in emulsion 2. Analysing the curves in Fig. 2, from the fact that curve *B*, except just at its origin, is a straight line, it follows that the reaction in emulsion 2 is proceeding at a practically constant rate throughout the experiment. This velocity is affected only to a very slight extent by the gradual decrease of the active mass of the oil. Curve *A*, on the other hand, shows a much greater initial velocity of reaction and a rapid progressive diminution of this velocity.

The factors responsible for the diminution of velocity, shown in curve *A*, are best considered from the mathematical standpoint. The mathematics of the reaction are extremely complex, but when reduced to their simplest form, they give some indication of what is occurring in the two emulsions during the experiment.

If two emulsions, in which the globules have the radius r_1 and r_2 respectively, contain equal amounts of oil in unit volume of emulsion, since the surface area of individual drops in them will be in the proportion r_1^2/r_2^2 and the number of drops in equal volumes will be in the proportion r_2^3/r_1^3 , the total surface of the drops in equal volumes will be in the proportion $r_1^2/r_2^2 \times r_2^3/r_1^3$ or r_2/r_1 , in this case 6/1.

During the first 15 min. (curves *A* and *B*, Fig. 2) the amount of oil hydrolysed in the two emulsions was equivalent to 26.2 and 4.4 c.c. $N/10$ respectively, a ratio of 5.95/1, practically the same as r_2/r_1 . The resulting reduction in the amount of unsaponified oil in emulsion 1 (9 p.c.) is greater than in emulsion 2 (1.5 p.c.), but not sufficient to account for the reduction in the rate of hydrolysis in the former (curve *A*). The other factor influencing the rate of hydrolysis is the diminution of surface presented by the oil drops which in emulsion 1 were observed to increase in size by coalescence throughout the experiment and gradually to approach the

size of the drops in emulsion 2, which did not change. If they actually attained that size the rate of hydrolysis would even become slower in

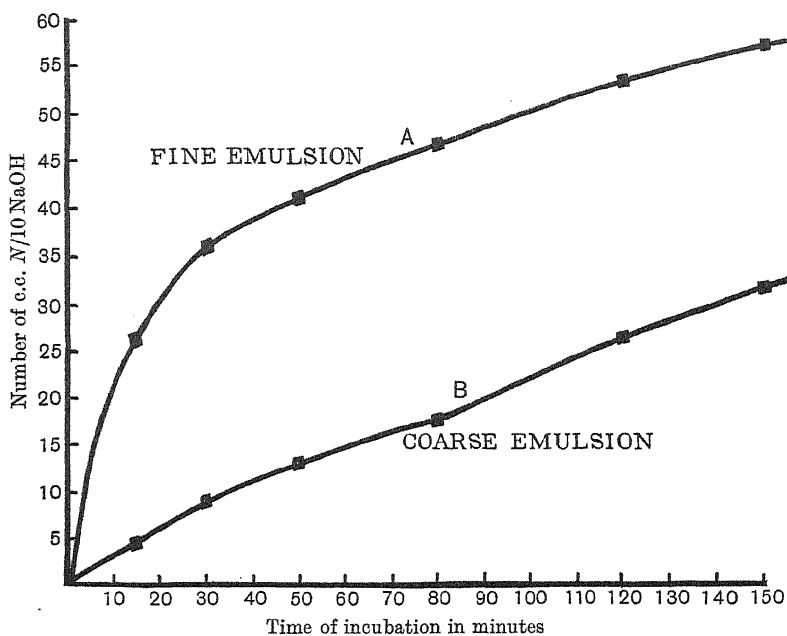


Fig. 2.

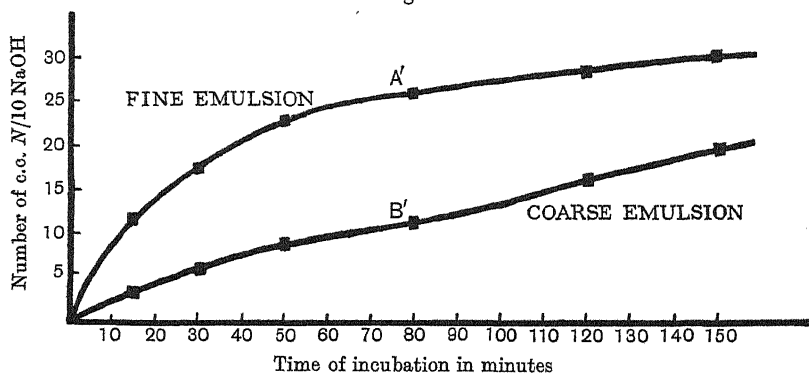


Fig. 3.

emulsion 1 than in emulsion 2 because the amount of unsaponified oil would be less owing to the increased rate of hydrolysis during the time when the drops were smaller and the total surface larger.

The straight part of curve *A* is practically parallel to curve *B* (the latter part of it actually somewhat less steeply inclined to the abscissa). This straight part represents the rate of hydrolysis when the drops have the larger size. If a straight line be drawn through the origin parallel to this straight portion, from 80 mins. on, and the ordinates from that line be subtracted from the corresponding ordinates of curve *A*, the curve *C* (Fig. 4) is obtained which represents approximately the rate of hydrolysis of fine drops alone and is similar to the curve that would be expected if the oil were in true solution. In the first 80 min. hydrolysis took place equivalent to 38 c.c. *N*/10 or 1.1 g. of oil, 13.7 p.c. of the amount originally present.

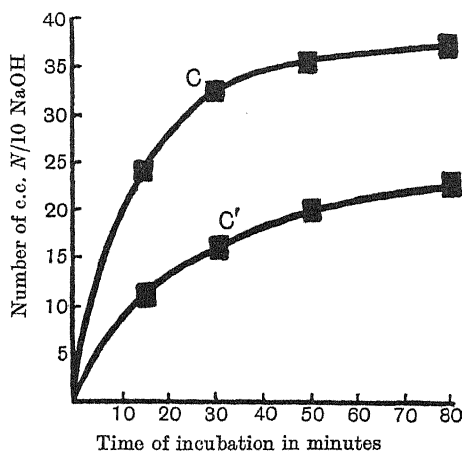


Fig. 4.

In Fig. 3 the curves were obtained from the second experiment in the second series. They are the same in form as in Fig. 2, but of smaller amplitude, since the emulsions used were only 10 p.c. oil in water. As in the case of curve *A* (Fig. 2) a curve *C'* (Fig. 4) was plotted. This shows that a slightly greater percentage of fat (16.7 p.c.) is being acted upon in the finely divided state in this experiment.

The pressure of 1000 lb./sq. in. used in these experiments for preparing the fine emulsions was not sufficiently high to yield completely stable emulsions. Emulsions have been obtained, operating with a pressure of 3000 lb./sq. in., which will not cream on incubating in an acid medium. Further experiments are being carried out with such solutions. These experiments will be the subject of a future communication.

SUMMARY.

An apparatus is described for the production of highly dispersed emulsions of oil in water, the particles of which are of the magnitude of $\frac{1}{2}\mu$ or less.

The rate of digestion of fat by lipase is proportional to the surface area exposed by the particles, and inversely proportional to the radii of the globules.

In a very finely divided emulsion the reaction curve approximates to that of a substance in true solution.

We wish to express our thanks to Prof. J. Mellanby for kindly supplying us with the lipase used during the first series of experiments, and to Prof. B. J. Collingwood and Prof. J. B. Leathes for their helpful assistance.

PROCEEDINGS

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February 18, 1933.

Calcium salts of bone. By CLARICE MARGARET BURNS.
(*Preliminary communication.*)

The estimation of the total acid soluble calcium and phosphorus in a large number of fat-extracted bones from rats and cats showed a Ca/P ratio of about 2.00, tending to 2.20 in older bone and falling as low as 1.85 in young bone. The percentage of the total calcium combined with carbonate ranges from 8-16, according to Kramer, *et al.* [1928]. It may then be calculated that in all except the old bone the $\frac{\text{residual Ca}}{\text{P}}$, *i.e.* $\frac{\text{Ca not combined with carbonate}}{\text{P}}$, must be less than the 1.94 required if all the bone calcium is to be present as tertiary calcium phosphate and calcium carbonate as concluded by Kramer. If the ratio $\frac{\text{residual Ca}}{\text{P}}$ is 1.81, this could be explained on the assumption that the calcium phosphate compounds were a mixture of 80 p.c. tertiary calcium phosphate and 20 p.c. CaRPO_4 , where R may be an inorganic or an organic radicle. These results were secured by examining adequate amounts of acid extracts of bone by standard macro-methods, *i.e.* precipitation of the calcium as oxalate, and estimation of the phosphate by either precipitating as MgNH_4PO_4 , and weighing as $\text{Mg}_2\text{P}_2\text{O}_7$, or by the volumetric ammonium molybdate method. Use of the micro-methods described by Kramer led to a considerable but variable loss of phosphate adsorbed to the precipitate filtered off after the addition of ammonium molybdate. This gave the higher Ca/P ratio found by Kramer. If, however, the method was modified by extracting with cold acid, producing the phosphate colour by Fiske's method [Fiske, 1925], and reading within 5 min. of adding the reagents, it was found quite possible to match the colours without the filtration Kramer found necessary by the Briggs' method. The results so obtained were about 2 p.c. lower than those obtained by the macro-methods, giving a much lower ratio than found by

Kramer. The method of bone analysis used by Morgulis [1931] was found to be inaccurate as the alkaline glycerol used to remove organic matter also dissolves out some phosphate, in growing bone, thus giving the high Ca/P ratio found by Morgulis in the mineral residue.

The presence of two types of calcium phosphate in growing bone may explain the different reactions of growing and old bone to madder. This is being investigated, as also the nature of the different compounds and their relative proportions in bone formed under different conditions.

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Indirect calorimetry applied to the fowl. By H. E. MAGEE and ERIC REID.

The mask sketched in Fig. 1 was designed to collect the expired air of fowls. It consists of a thin-walled rubber balloon (*a*) in which is inserted a rubber stopper (*b*) holding two one-way valves, inlet (*c*) and outlet (*d*), made of preserved rabbit intestine¹. Collapse of the wall of the mask during inspiration is prevented by thicker pieces of rubber (*e*) solutioned on to it. An opening (*f*) is made in the balloon to admit the fowl's head

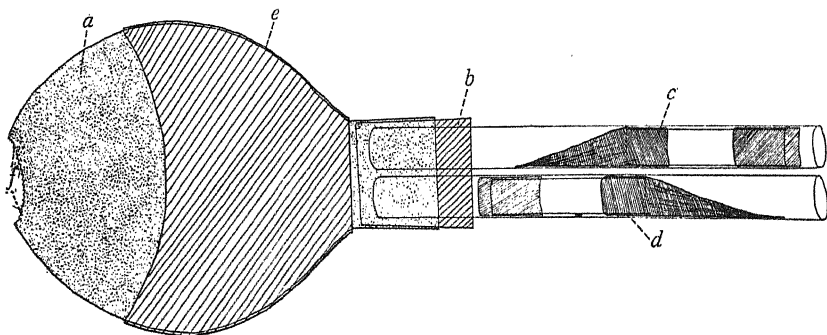


Fig. 1. Sketch of mask for fowl, half-size.

and such that the thin-walled rubber fits closely round the neck without impeding respiration. In a preliminary operation, the comb and wattles are removed so that the head fits into the mask closely and the dead space is reduced to a minimum. The feathers are stripped from the neck, thus ensuring close contact between the skin and the thin-walled rubber and, to further preclude leakage, vaseline is employed.

¹ These valves are of the same type as those described in Macleod's *Physiology and Biochemistry in Modern Medicine*, 6th edition, p. 783.

The mask is adjusted and the fowl allowed to breathe through it for 20 min. The outlet valve (*d*) is then connected to a Douglas bag of 20 litres capacity and the expired air collected for 15 to 25 min., analysed, and its volume measured. As the following experiment shows, a preliminary period of 20 min. is sufficient to annul the effects of over-breathing, which occasionally occurs when the mask is being fitted on. From a fowl, fasted for 24 hours, samples of expired air were collected 20, 60 and 120 min. after the mask had been adjusted. The values of the R.Q. were respectively 0.709, 0.710 and 0.699. After a week's training no difficulty was experienced in keeping the fowl perfectly quiet during an experiment.

The effects of fasting for periods up to 7 days and of ingestion of carbohydrates have been studied. In fasting the R.Q. fell from 0.95 in the fed state (mixed diet) to 0.69 in 24 hours, and varied between 0.69 and 0.64 until the end of the fast on the seventh day. It may be that the low R.Q. is related to the fact that uric acid is the chief nitrogenous end-product in the fowl. Ketone bodies were not found in the urine. The figures tabulated below show the effects of glucose ingestion.

Cock, 2.37 kg. fasted 24 hours; 25 c.c. 0.75 *M* glucose administered into gizzard by catheter.

Time after food (min.)	Expired air		R.Q.	Total heat production cals. per hr.
	p.c. CO ₂	p.c. O ₂		
0	3.53	16.17	0.69	6.98
25	4.08	16.18	0.80	7.46
50	4.14	16.35	0.87	7.41
125	3.76	16.08	0.72	6.64

These results show that the composition of the expired air of fowls closely resembles that of mammals and also that the effects of glucose ingestion on the R.Q. and on the heat production are similar in both types of animal.

The occurrence of Wedensky inhibition in myasthenia gravis.

By E. A. BLACK PRITCHARD.

Myographic records have been obtained from a patient suffering from myasthenia gravis: all voluntary movements were extremely weak, and rapidly fatigued to complete disappearance on attempted repetition. The ulnar nerve has been stimulated 4 cm. above the elbow through a surface electrode by discharging through the patient (the indifferent electrode being on the opposite forearm) a condenser of 1 μ F capacity charged at a potential of 18 volts. By means of a rotating commutator these condenser discharges have been applied at frequencies varying from 5 to

500 per sec. In order to secure effectively complete discharge of the condenser, the electrodes connected to the patient have been short-circuited through a resistance of 400 ohms: with this resistance present, the residual charge left in the condenser after the shortest discharge period used, is less than 1 p.c.; for all the lower stimulation frequencies the residual charge is still less than this. The forearm, hand, thumb and all fingers except the fifth finger have been immobilized by enclosing these parts in a plaster casing secured to the top of an experimental table. The fifth finger has been further splinted with light aluminium strips to prevent movement at any but the proximal, or metacarpo-phalangeal, joint. A wire connected the finger at the level of the last inter-phalangeal joint with the lever of a torsion myograph which carried a small mirror reflecting the image of an illuminated slit across the aperture of a film camera.

When records are taken in this way from a normal person, a single condenser discharge gives a single isometric twitch: a series of discharges at frequencies up to 500 per sec. gives a tetanic contraction in which the steeply rising curve of tension increase is followed by a horizontal plateau of maintained tension at the maximum height reached by the curve of tension increase.

In the case of the myasthenic patient the following results have been obtained:

- (1) No difference has been found between the simple twitch which follows a single stimulus in the myasthenic and in the normal subject.
- (2) Repeated single twitches fall off rapidly in height in the way well known to be a characteristic effect of this disease.
- (3) With brief (1-5 sec.) stretches of repetitive stimulation the curves of tetanic contraction which result show a change in general form as the frequency of repetition is increased. With low rates of repetition (40 per sec.) the curve is similar to that obtained from a normal control, *i.e.* the plateau, although a little irregular from incomplete tetanic fusion, is maintained at the maximal level originally reached by the curve of tension increase. When the frequency of repetition is increased to 80 per sec., the plateau is maintained at less than this maximal height, *i.e.* after the initial steep rise the curve falls slightly before becoming horizontal. As the frequency is progressively further increased, the maximal height initially attained remains unaltered, but the drop to the plateau becomes progressively greater, *i.e.* the plateau forms at a progressively lower level until with a frequency of 500 per sec. it is only just above the original base line and less than $\frac{1}{3}$ of the height of the initial contraction.

The fact that with increasing frequencies the plateau forms at decreasing heights is not due to decreasing strengths of the stimuli consequent upon incomplete charging or discharging of the condenser: the resistance in the charging circuit—and hence the time taken to reach the maximum charge—is negligible: the presence of the short-circuiting resistance of 400 ohms in the discharging circuit ensures that even with the maximum frequency of stimulation more than 99 p.c. of the charge leaves the condenser. Therefore, it is suggested that this is an example of Wedensky inhibition occurring in the ulnar nerve—finger flexor preparation of a human subject.

A mouthpiece for collecting expired air in dyspnœa.

By R. HILTON.

The mouthpiece was made in order to obtain expired air from patients with dyspnœa, or from healthy persons during exercise. An attempt has been made to diminish the resistance to breathing (especially in the expiratory valve) and also to reduce the dead space. The apparatus is simple and cheap to make and can easily be cleaned. It consists of a piece of stout glass tubing 12 cm. long and 3 cm. in diameter. Near the middle

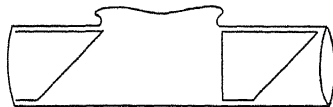


Fig. 1.

is an oval opening with an everted lip, 4 by $1\frac{1}{2}$ cm., round which fits a rubber band for the teeth to close on. An oval piece of thick rubber sheeting stretched round the everted edge fits between the lips and teeth and prevents all leakage. The rubber valves are carried on two identical pieces of thick glass tubing $4\frac{1}{2}$ cm. long, which are each cut slanting at an angle of 45° at one end. The outer diameter is $2\frac{1}{2}$ cm., the inner is 2 cm. Thin rubber sheeting is cut to cover the two slanting ends, and is stuck lightly to the glass with seccotine. The diameter of the two slanting tubes as given is small enough to fit easily into the main piece. The leakage of air between is prevented by binding rubber on the inner tube. French chalk is rubbed on the valve flap and the glass edges with which it comes into contact in order to prevent sticking from condensed moisture. It is to be noted that the valves are not meant to work upside down. They are put into such a position that their closure is aided by gravity.

In use the mouthpiece is more comfortable than the standard types

made of metal. It does not show any measurable leak if the valves are made of good rubber. The action of the valves is quiet though audible during vigorous breathing.

A comparison has been made between the new mouthpiece and two standard valves—the spear head type and the circular rubber valve working on a perforated metal plate. The resistance of all three has been measured by a water manometer at rates of flow of gas varying from 5 to 20 litres per min.

The following graph shows that the mouthpiece has a lower resistance than the other two types in common use.

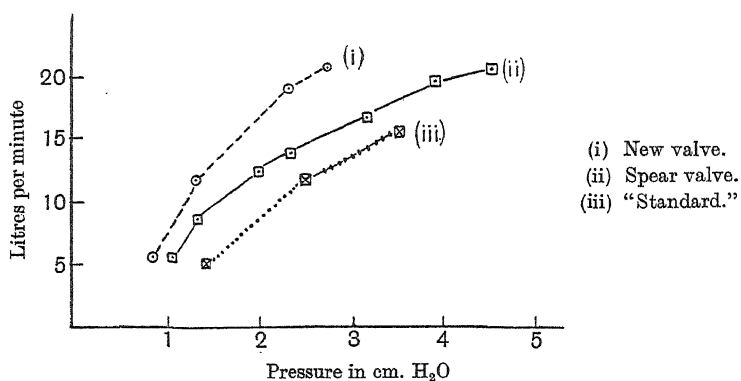


Fig. 2.

The measurements of the dead space are as follows:

- | | |
|-------------------------------------|-------------------|
| (i) The new mouthpiece | 33 c.c. (approx.) |
| (ii) The spear valve | 100 „ „ |
| (iii) The circular "standard" valve | 43 „ „ |

These figures appear to show that the diminished resistance has not been obtained at the expense of an increase in the dead space.

An essential growth factor for the anaerobe *B. sporogenes*.

By B. C. J. G. KNIGHT and PAUL FILDES. (*London Hospital*.)

As basal medium we have used an acid hydrolysate of gelatin with the addition of tryptophane, cysteine, the usual salts, and KH_2PO_4 to give a final concentration of $M/30$, the pH being adjusted to 7.2.

On this medium, under anaerobic conditions, *B. sporogenes* will not grow from washed spores. Occasionally a faint growth is obtained and nothing further after several days. It is therefore not a question of rate of

growth in these cases, but of some limiting factor. This we believe is due to traces of the factor we are to describe, which are sometimes present as inadvertent impurity.

With the above basal medium we have obtained normal growth by the addition of highly dilute solutions of an active preparation. The original source of this active preparation was pressed baker's yeast, chosen for convenience, although the active substance is widely distributed in animal and vegetable tissues.

The yeast was dried at 50° C., ground and extracted with successive quantities of boiling aqueous ethyl alcohol (75 p.c.). The fatty mixture from this extraction was dried down with plaster of Paris, and ether-extracted (Soxhlet). The ether extract was acetone precipitated, the activity remaining in solution. The solvents were removed and the residue saponified with dilute alcoholic soda. The alcohol was boiled off and the aqueous alkaline solution ether-extracted. The ether extract was inactive, the activity remaining in the aqueous layer. The soda solution was saturated with CO₂ and again ether-extracted. The activity remained in the aqueous layer, which was then acidified (H₂SO₄) and thoroughly ether-extracted. Most of the activity was now found in the ether, indicating that the activity was due to an acidic substance. The partition coefficient was evidently not very large.

Fatty acids were precipitated from this acid fraction as barium soaps, the activity remaining in the filtrate.

At this stage the product was about 1 g. of light yellow gum. 2.0×10^{-7} g. (1/5,000,000 g.) of this product in 10 c.c. of the basal medium will cause normal growth. From a very rough estimate the active quantity per organism is of the order of 1/20,000 of the weight of one bacillus, and the product is as yet by no means pure.

As so far purified the preparation has the following properties: it does not contain nitrogen, sulphur or halides; is easily nitrated (xanthoproteic reaction); gives a positive Millon reaction; easily reduces alkaline neutral, and acid potassium permanganate in the cold; ferric chloride gives a yellow colour.

Oxidation by permanganate or hydrogen peroxide destroys its activity. In the later stages of the separation the positive Millon test and easy reduction of permanganate paralleled the activity, indicating that these reactions possibly concerned the active substance and not associated impurities.

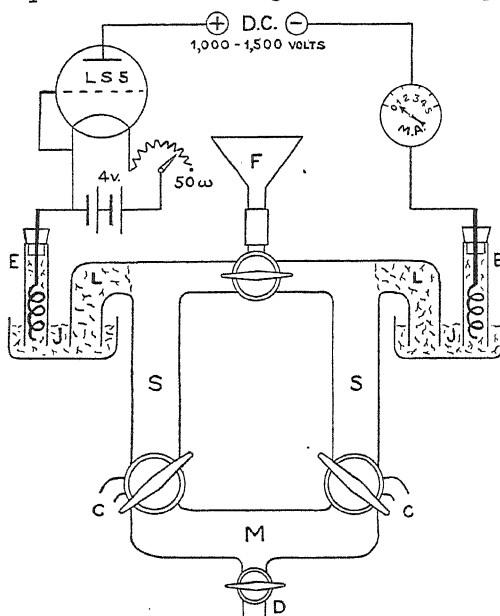
At this stage the close similarity of properties with the "auxin" of Kögl and Haagen Smit, which produces increase of length of plant

cells, was recognized¹. Consequently we looked for our active substance in urine (pregnancy and male) and found it in the same fraction of the saponified ether extract as Köggl finds his "auxin."

Our preparation from the same source is now being further purified to see whether the parallelism with "auxin" remains, and to obtain our essential factor for *B. sporogenes* in a state of purity. The possibility of its being required by other bacteria is being studied.

Electrophoresis apparatus. By D. T. HARRIS.

This is the usual cataphoresis U-tube specially modified for the repeated and rapid collection of charged material during prolonged and



E, non-polarizable electrode, e.g. Ag/AgCl; *J*, junction vessel; *L*, side-limb or connecting arm; *E*, *J*, *L*, filled with saline + 2 p.c. agar; *S*, vertical tube containing the suspending medium at appropriate pH; *M*, complex mixture to be separated; *F*, safety funnel kept full of fluid for maintaining *S* when *C* in use; *D*, drain, connected by rubber tube to filling funnel; *C*, collecting tube for drawing off component which has migrated into *S*.

continuous working. It will be found useful for dealing with such complex mixtures as are found in tissue extracts, vitamin concentrates, viruses or immune bodies².

¹ Koninkl. Akad. Wetensch. Amsterdam, *Proceedings*, 34 (1931), p. 1411; *Die Umschau* (Frankfurt a.M.), 40 (1932); *Naturwissenschaften*, 21 (1933), p. 17.

² Manufactured by Messrs Baird and Tatlock.

In order to avoid the deleterious effects of electrolysis and heating and yet obtain a high migration velocity, it is necessary to use a low current density in conjunction with a strong directional field. For practical purposes a current density not exceeding 1 milliamp. per sq. cm. and a P.D. of 10-50 volts per cm. are desirable. The former may be realized by using a tube of wide bore and a current-limiting device, *e.g.* a thermionic valve with filament considerably under-run, while the latter can only be achieved by keeping the electrolyte content of the suspending medium at a minimum and is limited by conditions such as *pH*. The E.M.F. necessary may be obtained from cheap dry batteries or from the mains: (i) A.C. with step-up transformer and rectifying valve, (ii) D.C. with a small motor generator, each calculated to deliver 5-10 milliamp. at 1500 volts.

Intrarenal pressure. By F. R. WINTON.

An increase of ureter pressure does not retard the urine secreted in a heart-lung-kidney preparation unless the pressure exceeds a definite value, usually about 10 mm. Hg [Winton, 1931 *a*]. An increase in venous pressure, less than about the same value, is likewise without effect on the urine flow [Winton, 1931 *b*]. The creatinine content of the urine secreted against a ureter pressure within this ineffective range is independent of the pressure.

These observations can be explained by supposing that even in the unobstructed kidney there is a pressure, exerted in all directions throughout the substance of the organ, tending to obliterate collapsible structures such as the peripheral parts of the tubules and the venules. Such an *intrarenal pressure* would retard the urine flow by means closely similar to those known to operate when urine flow is reduced by increase of venous pressure, that is, the compression of the distal parts of the tubules by the distended venules. In partial venous obstruction, increase of ureter pressure has no effect on the urine flow unless the ureter pressure exceeds the venous pressure [Winton, 1931 *b*]. Similarly in the unobstructed kidney, increase of ureter pressure will be without effect unless its value exceeds that of the intrarenal pressure.

In support of this conception, it is noteworthy that the critical value which must be exceeded if ureter or venous pressure is to affect the urine flow is about the same for both in the same kidney. Moreover, the fact that the ureter or venous pressure needed to reduce the urine flow a given amount is disproportionately greater than the fall of arterial pressure

which would reduce it by the same amount [Winton, 1931 *a*, *b*] is readily explained by the hypothesis of the intrarenal pressure.

The quantitative significance of the intrarenal pressure varies greatly in different kidneys. In some organs it cannot be detected; in most isolated kidneys, however, its value appears to lie between 4 and 14 mm. Hg. In a typical kidney the urine flow is unaffected by a ureter pressure of 10 mm. and abolished by a pressure of 20 to 30 mm. One may estimate that the urine flow in such an unobstructed kidney would be 50 p.c. to 100 p.c. greater if the intrarenal pressure could be removed.

Preliminary observations suggest that the intrarenal pressure may be raised by the presence of high concentrations of certain diuretics in the blood; 0.4 p.c. sodium sulphate, for example, raised it in one kidney from 8 mm. to 22 mm. Hg. Urea and glucose have a similar but less marked effect. Increase of arterial pressure, however, sufficient to produce a comparable degree of diuresis may have no effect on the intrarenal pressure.

The polyuria of one half of a kidney, induced by ligation of the branch of the renal artery supplying the other half, may be due to a reduction of the intrarenal pressure associated with the change from a relatively tense to a flaccid condition of the kidney. The release from a tonic inhibitory influence which Verney [1929] postulated to account for this polyuria could thus be explained without invoking the nervous action on the glomerular pressure which he suggested.

It is difficult to assess the importance of intrarenal pressure in the normal kidney *in situ*, for though a moderate increase of ureter pressure does not reduce the urine flow, and may increase it, this may be due to a reflex change in the glomerular pressure. Since, however, an enhanced intrarenal pressure largely reduces or even abolishes the urine flow, it is likely to be a significant factor in the behaviour of diseased kidneys, especially those associated with engorgement or cedema.

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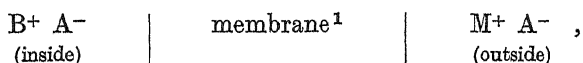
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Stethographic method to ensure that alveolar air samples are taken in the correct phase of respiration. By CHARLES E. BRUNTON and M. C. G. ISRAËLS. (*Dept. of Physiology, London Hospital Medical College, and Manchester Univ.*)

By using blood-pressure armlets as stethographs, one on the chest and one on the abdomen, respiratory tracings may be obtained from human subjects. From these it is possible to check the phase of respiration in which Haldane-Priestley samples are given. A sample given at the wrong phase can be rejected without analysis and be replaced by another. If the subject is sitting and leans forward slightly to deliver the sample, a false inspiratory stroke may be produced on the thoracic curve. It may be distinguished from a true inspiration by the angle which it makes with the base line, or the movement of the subject's body may be noticed by an observer and separately recorded if desirable. The method is useful with untrained subjects.

Remarks concerning ionic distribution during membrane diffusion. By TORSTEN TEORELL. (*Caroline Institute, Department of Physiological Chemistry, Stockholm.*)

If we consider the system



BA and MA being strong electrolytes, and beginning at the same concentrations, the following is evident: (1) If the cations B^+ and M^+ are freely diffusible through the membrane the concentrations of B^+ , M^+ and A^- respectively must be identical on each side of the membrane, provided that a sufficiently long time has elapsed from the start of diffusion. (2) If the mobility of B^+ in the membrane = 0, the typical Donnan effect will appear, *i.e.* $\text{A}_1 > \text{A}_0$. (3) If the mobility of B^+ is small in comparison with that of M^+ there will arise a "relative" or "incomplete" temporary Donnan effect on the ionic distribution. A short time from the start of the diffusion A_1 will be greater than A_0 ². Finally however the diffusion restores $\text{A}_1 = \text{A}_0$ (if the system is not subjected to factors disturbing the

¹ The membrane may be of "sieve" type or another phase.

² The osmosis of water being neglected.

equilibria as chemical reactions). The following table is taken from one of the author's experiments:

TABLE.

"*Inside*": a small parchment tube filled with 2.5 c.c. of 0.160 molar NaCl. The free surface of the liquid in the tube was always adjusted in level with the surface of

"*Outside*": a beaker containing 1000 c.c. of 0.160 molar HCl.

Convection on both sides by means of a stream of air bubbles.

Acidity determined on 0.1 c.c. by micro titration (phenol red), chloride determined in the same sample electrometrically.

The concentration figures expressed in millimols per litre (activity figures would of course be more correct).

Minutes from start of diffusion	Acidity (inside)	Chloride (inside)
0	0	163
15	41	173
30	71	185
45	95	191
60	109	193
75	123	195
90	127	191
120	144	191
150	152	185
180	155	181
210	155	172

Outside (120 min.) { Acidity 160
Chloride 161

The table shows that there was an anion accumulation in the parchment "cell" during the first stages of diffusion, *i.e.* there was a diffusion against the osmotic concentration gradient. With the positions of HCl and NaCl reversed there was a chloride decrease inside.

The bearings of these observations on physiological ionic distribution, especially in connection with the composition of the gastric juice, will be communicated later on. Experiments performed by the author seem to show that hydrogen ions can penetrate the gastric mucosa in the direction from the juice to the blood. An attempt to explain certain relations between acidity, secretory speed and chloride content of the gastric juice will also be given.

PROCEEDINGS

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March 18, 1933.

Automatic pressure control. By S. J. FOLLEY and G. L. PESKETT.
(*National Institute for Research in Dairying, Shinfield, Reading.*)

Numerous forms of apparatus have been suggested for the control of pressures, both "negative" and "positive," in the laboratory. That which is described below has the advantage of providing a very constant pressure while being simpler and less expensive to construct than many others. It is being used by one of us for ultra-filtration at a pressure of 150 mm. Hg, but its principle should be capable of application to a wide range of pressures.

The apparatus, shown in the diagram (Fig. 1), is based on that of Miller and McKinney [1928] who, however, used it for partial

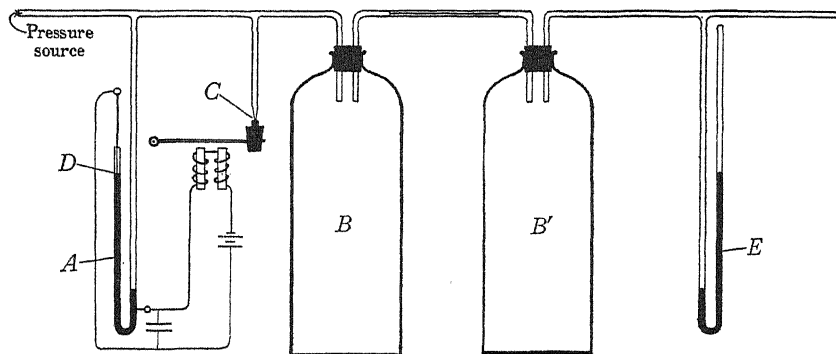


Fig. 1.

vacua. The regulating manometer *A* is provided with two platinum wires; one sealed into the lower limb, the other capable of adjustment to the required level. For the latter purpose a rack and pinion movement could be used. *A* is sealed into the side of the glass tube leading from the source of pressure (in our case a cylinder of compressed air or nitrogen) to the buffer reservoirs *B* and *B'*. These are connected by means of a glass capillary of bore 1 mm. and length 150 mm. which offers resistance to the passage of pressure fluctuations from *B* to *B'* so that the pressure in *B'* is practically constant. The platinum wires are connected, as shown, with an electric circuit which includes a condenser ($4 \mu\text{F}$) to

reduce sparking at the mercury-platinum contact. The circuit operates an electromagnet, on the arm of which is mounted a cork carrying a small stopper of soft rubber. This stopper opens or closes a fine nozzle at *C*, which acts as a valve, opening as soon as the pressure rises above that desired, and closing when the pressure falls sufficiently to break contact at *D*. The arm of the electromagnet should have a strong spring and should not allow more than 2 mm. movement.

The efficiency of the apparatus may be judged from the fact that we have scarcely been able to observe any movement of the meniscus in the manometer *E*, even when using a lens, so we may conclude that the pressure variations do not exceed 0.1 mm. Regarding ease of construction it may be remarked that our apparatus was made and assembled in a few hours using an old bell for the valve control and two Winchester quart bottles for the reservoirs. It seems that the barostat control of Miller and McKinney is as useful for higher pressures as they claimed for it in the case of partial vacua. It is suggested that it can be used to secure accurate control in several physiological and chemical operations, *e.g.* perfusion, intratracheal anæsthesia, metabolic studies of small animals, ultra-filtration, vacuum distillation, gas sorption and others where a constant gas pressure is required.

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The influence of temperature on the isolated mammalian kidney. By R. G. BICKFORD and F. R. WINTON.

Cooling a kidney practically abolishes secretory activity and provides a preparation in which the filtration mechanism can be studied. The effect of cooling is reversible within the range examined, namely 3° C. to 38° C.

If an isolated canine kidney be perfused with defibrinated blood of the same composition, alternately, from a pump-lung circulation at 37° C. and from a similar circulation at a variable temperature, the consequences of cooling can be dissociated from the changes which occur spontaneously in the surviving organ, and can, therefore, be expressed in quantitative terms. Cooling through the first 20 degrees below body temperature has a relatively great effect, whereas cooling through a further 15 degrees has a relatively small additional effect; the results of a series of such experiments will therefore be summarized by describing the effects of "cold" in terms of those of a fall of 25° C.

The urine flow is increased by cold; at a flow of about 4 c.c. per

10 min. in the warm kidney the increase is about threefold; at a higher rate of flow induced by higher arterial pressure the increase is only about twofold; at lower rates the increase becomes progressively greater till it reaches infinity when the pressure is low enough to prevent urine flow in the warm kidney. If the urine flow in the warm kidney be abolished by reducing the arterial pressure (*e.g.* to 70 mm. Hg), cooling the kidney induces a considerable flow at the same pressure, while rewarming the kidney again abolishes the flow. To prevent formation of urine in such a cold kidney the arterial pressure must be lowered at least to 40–50 mm. Hg.

The following observations indicate that the composition of the urine produced by the cold kidney approaches that of a plasma-transudate. The low chloride concentration characteristic of the urine of the warm isolated kidney is replaced in the cold kidney by a concentration equal to that in the plasma. The ratio of the concentrations of creatinine in urine and plasma, which may be about twenty in the warm kidney, approaches unity in the cold kidney, but, as already stated, the twentyfold increase of urine flow which would seem to be demanded by the filtration-reabsorption hypothesis does not occur.

The absolute quantity of chloride excreted in a given time is greatly increased by cold, but, at normal arterial pressures the absolute excretion of creatinine is reduced. If activity of the tubules be abolished by cold, the filtration-reabsorption hypothesis might lead one to anticipate that the absolute creatinine excretion would be unchanged, whereas the secretory hypothesis would demand just such a reduction as is obtained. The comparable effect of cyanide in abolishing tubular activity results in a similar reduction in the absolute rate of excretion of urea, and this has been claimed as evidence of secretion in the tubules [Starling and Verney, 1925]. Cyanide similarly reduces the absolute excretion of creatinine, but Bayliss and Lundsgaard [1932] prefer to retain the filtration-reabsorption hypothesis, and to attribute the reduction to the loss of creatinine in tubules rendered permeable by poisoning. Cold, however, is unlikely to produce a reversible increase in the permeability to creatinine of the tubule walls, and since cold and cyanide reduce the output of creatinine under the same conditions, Bayliss and Lundsgaard's explanation is rendered improbable.

One would seem, therefore, to be driven to adopt the secretory hypothesis of Starling and Verney, were it not for the fact that, at low arterial pressures, cold increases the absolute rate of creatinine excretion, although at normal or higher pressures it has the opposite effect. This

increase in the creatinine output due to cold at low pressures, under conditions favouring the highest concentration ratios in the warm kidney, is difficult to explain on the secretion hypothesis. On the filtration-reabsorption hypothesis it would indicate that cold reduces the leakage of creatinine from the distal part of the tubules; there are other reasons making the existence of such a leakage a necessary consequence of the hypothesis.

The reduction of creatinine excretion due to cold at higher pressures may tentatively be attributed to a reduction of filtration pressure in the glomerulus associated with the extreme polyuria; this may well be due to increase in viscous resistance in the collecting tubules, partly due to increased velocity of flow, and partly to the increase of viscosity at the low temperature. Cold produces well-marked and reversible swelling and increase in tenseness of the kidney, which suggests an accompanying increase in intrarenal pressure and consequent reduction in the rate of glomerular filtration.

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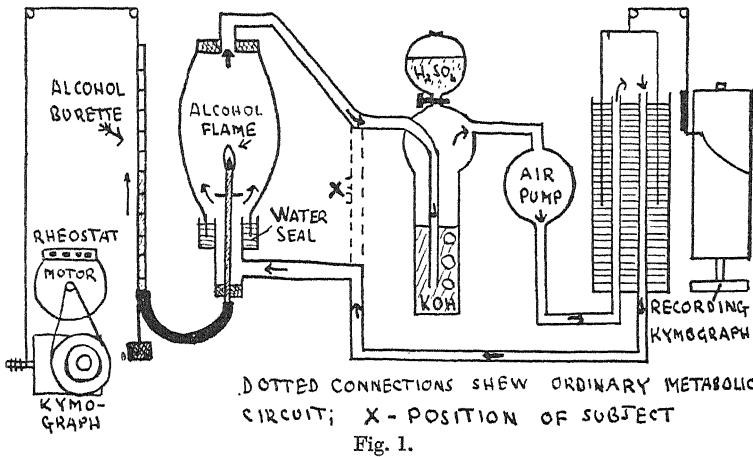
A simple alcohol check apparatus for closed circuit metabolic work. By A. A. MONCRIEFF¹ and A. T. WILSON. (*From the Department of Physiology, Middlesex Hospital.*)

Basal metabolism in man and animals is commonly investigated by some form of closed respiratory circuit in which oxygen consumption and CO₂ production are measured. Apparatus for this purpose is open to a number of sources of error difficult to detect other than by alcohol checks.

In these the experimental subject is replaced in the closed air circuit by an alcohol jet burning a known volume of pure ethyl alcohol—sp. gr. 0.792 at 20° C. One gramme of this requires for complete combustion 1458 c.c. of O₂ and produces 972 c.c. CO₂ (R.Q. .67). The theoretical O₂ consumption and CO₂ production of the alcohol is afterwards compared with the actual figures registered by the apparatus during this controlled combustion. In practice, errors of more than 3 p.c. may thus be avoided. Various complex forms of alcohol check have been described. The simple device illustrated here was evolved after considerable experiment and has been found to work satisfactorily.

¹ Working with a part-time personal grant from the Medical Research Council.

Fig. 1 shows how the alcohol combustion chamber is inserted in the air circuit of the metabolic apparatus. Fig. 2 shows in more detail the structure of the base of the combustion chamber with the alcohol jet.



This is made of pyrex glass (bore 1 mm.), and is enclosed in a water-sealed glass chamber with a metal base which admits the circulating

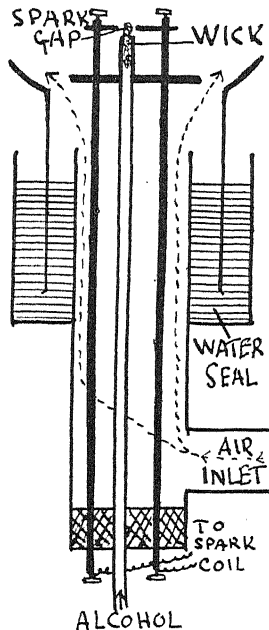


Fig. 2. Base of alcohol combustion chamber.

air, the alcohol jet and two copper rods which form at their upper ends an adjustable spark gap over the end of the alcohol burner and close to the small wick placed there, composed of 10 mm. of autoclaved asbestos string. The spark, used to ignite the alcohol flame, is supplied from a Ford ignition coil—a suggestion originally made by Mr Ellis of Messrs Palmer. The alcohol is fed to the jet by slow and regular raising of a burette, by means of a student's kymograph turned at any required speed by a rheostatically controlled electric motor.

In practice the air circulating pump of the metabolic apparatus is started and readings of levels on alcohol burette and gasometer are taken. The alcohol is then ignited by the spark, and burns at a rate, and in an amount, controlled by the rheostat of the elevating motor and by the kymograph gearing, such as to parallel the O_2 consumption when the metabolic apparatus is in ordinary use. The CO_2 absorbed by KOH is afterwards blown off by H_2SO_4 . Corrections are made for CO_2 content of KOH, volume of fluid added, temperature and pressure.

An anti-diuretic substance extracted from the liver.

By G. W. THEOBALD and M. WHITE.

It was found that 10 c.c. of an extract from ox liver when injected subcutaneously inhibited water diuresis in bitches for about 50 min. even though diuresis had commenced before the injection was made. The extract was prepared in the following manner. The liver was finely minced and three times the weight of water added. The proteins were coagulated at pH 4.5 at $80^\circ C$. The filtrate was concentrated under reduced pressure (25 mm. Hg) and neutralized to pH 5 (ca.). Fractionation with 75 p.c. alcohol in two stages was then carried out and the final filtrate was kept in cold store and freed from precipitated protein. This protein-free filtrate was used after the alcohol had been distilled off under reduced pressure.

The substance has a small molecule since it can pass through a collodion bag. It is less stable in a water than in an alcohol solution, is destroyed by boiling and would appear to be fairly easily oxidized. The extract, besides inhibiting water diuresis, causes contraction of the guinea-pig's uterus and constriction of its bronchioles, while it lowers the arterial blood-pressure of an etherized cat. Moreover it gives the bromine colour reaction characteristic of histamine and allied substances. It was therefore evident that the extract contained histamine, and it remained to be determined whether the amount of histamine present in 10 c.c. of the liver extract was sufficient to inhibit water diuresis. By comparing

the fall in blood-pressure in an etherized cat caused by the intravenous injection of 0.01 mg. of histamine with that caused by different amounts of the liver extract, it was evident that 0.2 c.c. contained less and 0.3 c.c. contained more than the standard amount of histamine. It was subsequently found that 0.5 mg. of histamine in 10 c.c. of normal saline solution when injected subcutaneously did not inhibit water diuresis. Further, all the samples of liver extract tested had approximately the same histamine content although most of them were no longer capable of inhibiting water diuresis. In an attempt to remove the histamine HNO_2 was added to the extract in the cold. The resulting solution had a much more powerful anti-diuretic action, 5 c.c. sufficing to inhibit diuresis for nearly 3 hours, although it had very much less effect on the blood-pressure of the etherized cat. It would therefore appear probable that the liver extract contains an anti-diuretic substance in addition to the histamine.

Further observations upon the action of œstrin on the isolated uterus. By G. F. MARRIAN and W. H. NEWTON. (*University College, London.*)

Several experiments of the following type have been performed. Two mature female guinea-pigs, weighing from 350 to 400 g., were anaesthetized with ether, and one uterine horn removed from each. These horns were suspended in a bath of physiological saline, as previously described [Marrion and Newton, 1932], and their spontaneous contractions recorded. One of the animals was then injected subcutaneously with a solution of pure crystalline ketohydroxy-œstrin over a period of 48 hours, and the other with a similar amount of the solvent. The total amount of œstrin given amounted to 0.6 mg., which represents about 25 c.c. of fluid; the injections were given hourly, except through the night. 18 to 24 hours after the last injection, both animals were killed by a blow on the head, and their remaining horns removed and records taken as before. The two horns of the control animal gave, as a rule, similar tracings on the two occasions, and such variations as were met with were inconstant. On the other hand, in spite of the wide diversity in type of the tracings from the first horn of the œstrin-injected guinea-pigs, those obtained from the second horn showed a great similarity. The contractions were relatively infrequent, regular, and apparently maximal, minor contractions being almost or entirely absent, and the muscle relaxed fully between the beats.

Numerous other experiments of a different nature have been done, using guinea-pigs and mice; they may be divided into three groups.

(1) Tracings of isolated uteri during œstrus and diœstrus have been compared.

(2) The uterine horns of an animal have been put up, one in the plain saline solution, the other in similar solution in which ketohydroxy-œstrin had been dissolved, so that the amount in the bath was 0.2 or 0.4 mg.

(3) The same as (2), except that both horns were in plain saline, and after a short normal tracing had been taken, œstrin (in alcohol) was added to one bath, and the same amount of alcohol to the other. Doses of 0.2–0.6 mg. of œstrin were employed.

The tracings from these experiments, considered as a whole, show a slight tendency on the part of the horns treated with œstrin, or taken from œstrous animals, to give the more regular type of contraction just described, or to exhibit less spontaneous activity and remain more relaxed than their controls. However, this does not apply to every case, and the only admissible conclusion is that the effect of œstrin *in vitro* is negligible; neither does there appear to be any correlation between the type of contraction and the presence or absence of œstrus, but further experiments are desirable to elucidate this point completely.

It seems, therefore, that while œstrin has little or no effect if applied directly to the uterine muscle, it is capable of profoundly modifying its contractions when administered some time previously to the intact animal. (That a similar modification is not obvious during natural œstrus suggests that the dose must be excessive.) This fits in with some observations of Azuma and Soskin, who found that œstrin, even when given intravenously, had no immediate effect on the uterine contractions of dogs, although these were depressed 24 hours later. Œstrin evidently acts indirectly, by undergoing some chemical change in the body, by merely causing hypertrophy of the muscle fibres, by altering the endocrine balance, or in some other way. This, however, does not explain our previously reported finding, namely, that œstrin, *in vitro*, has a definitely depressing action on certain types of contraction initiated by oxytocin. It may be that the activity induced by oxytocin is abnormal in character, or that the simultaneous presence of œstrin and oxytocin in the bath introduces some physical or chemical factor unrelated to the physiological action of either of the hormones.

The expenses of this and the previous work were defrayed by grants from the Medical Research Council.

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PROCEEDINGS

OF THE

PHYSIOLOGICAL SOCIETY,

May 13, 1933.

Oxygen content, capacity and percentage saturation in arterial blood at Johannesburg (altitude 5750 feet). By A. DIGHTON STAMMERS. (*Univ. of the Witwatersrand, Johannesburg, S. Africa.*)

The following are results of investigations carried out with van Slyke's apparatus with closed manometer. Duplicate estimations were made in every case, with negligible error.

Case	Oxygen content	Oxygen capacity	Percentage saturation
1	17.7	18.9	93.7
2	20.4	21.9	93.2
3	17.5	18.9	92.6
4	17.9	19.0	94.2
5	18.8	20.2	93.1
6	17.7	18.9	93.7
7	18.1	19.6	92.3
8	17.6	18.9	93.1
9	17.4	18.7	93.0
10	20.3	21.7	93.6
11	18.9	20.4	92.7
12	17.2	18.5	93.0
13	18.9	19.4	97.4
14	21.4	22.1	97.0

In the first ten cases the blood was taken from the brachial artery at the elbow. In Cases 11 and 12 it was taken from the veins at the back of the hand (technique of Goldschmidt and Light [1925]).

In Cases 13 and 14 it was taken from the brachial arteries of hospital patients after operation (Case 13, appendix; Case 14, carcinoma), the anæsthetic used in each case being nitrous oxide and oxygen.

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Human electrocardiograms recorded with the cathode ray oscillograph. By BRYAN H. C. MATTHEWS. (*Physiological Laboratory, Cambridge.*)

Rijlant [1931, 1932] has recently published human electrocardiograms taken with a cathode ray oscillograph. The sketch in A shows the general shape of the curves produced by his apparatus. He reports the

presence of a large slow S wave (S_2) and a secondary inverted T wave (T_2); he suggests that these are of physiological origin.

Such waves have never appeared in any oscillograph record of an

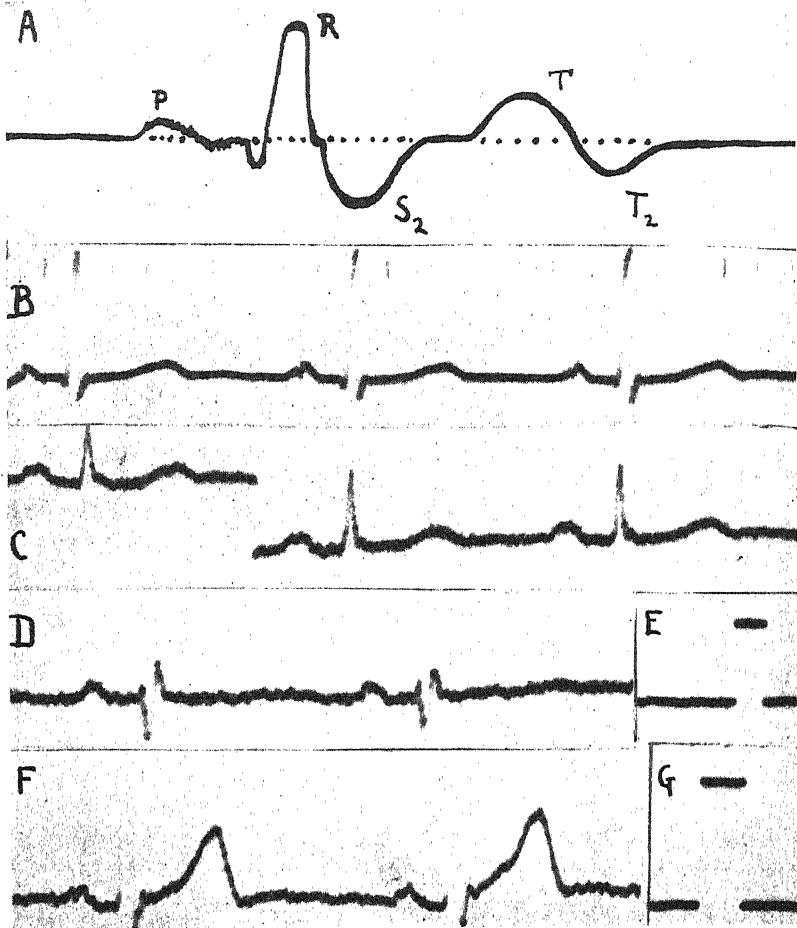


Fig. 1. Human electrocardiograms. A. Sketch to show type of curve recorded by Rijlant. B-G. Records taken in Cambridge with a Cossor cathode ray oscillograph and three stage battery coupled amplifier. B, C, D. Leads I, II, III, from the same subject. Calibration in C 1 millivolt applied in series with electrodes. E. Direct calibration 1 millivolt. F. Lead II, another subject with greater amplification. G. Direct calibration 1 millivolt. Time marker in B shows 0.1 sec. intervals.

electrocardiogram taken in Cambridge, and there seemed no reason why they should not have been observed many years ago in string galvano-

meter records. Having a Cossor cathode ray oscillograph set up for taking continuous records and a three stage battery coupled amplifier available in Cambridge, it seemed worth while to record a few human electrocardiograms with this combination to trace the origin of Rijlant's unusual curves.

Specimen records obtained in this way are shown below together with calibration curves of the apparatus, the amplifier is as nearly distortionless as it is at present possible to make an amplifier. The records are essentially like the classical string records; there are no large slow S_2 or T_2 waves even if considerable amplification be used.

The configuration of Rijlant's curves appears to be due to his amplifier rather than to any peculiarity of his subjects or the electrodes he uses; this is proved by his own experiments in which he takes simultaneous records with a string galvanometer from common electrodes; the string shows no sign of the slow S_2 or T_2 waves but they are of very considerable amplitude in the oscillograph record; had corresponding potential changes occurred at the electrodes there is no reason why the string should not have recorded them; they must therefore have been generated in the amplifier en route to the oscillograph.

It is impossible to say to what in Rijlant's amplifier the distorted S and T waves are due as he does not publish calibration curves of his apparatus or exact details of the amplifier, but as he states that the R waves in his records are often flat-topped owing to overloading of the output valve and he is also using an extensively decoupled resistance capacity amplifier there are plenty of opportunities for such distortions to be introduced.

Curves, showing slow S_2 and T_2 waves very like those published by Rijlant, have been recorded when the battery coupled amplifier is coupled to the cathode ray oscillograph through a capacity resistance distorting circuit of short time constant.

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Some estimations on the blood and urine of the 1933 Cambridge boat-race crew. By J. W. THORNTON and E. G. WHITE. (*University College, London.*)

Samples of urine were collected from each member of the crew on three occasions before the race, and two after, namely: (1) just before retiring on the night of March 30th; (2) on rising on the morning of the

31st; (3) immediately after the light morning's exercise in the boat about 11 a.m. on the 31st; (4) about 15 minutes after the race on April 1st; and (5) about $1\frac{1}{2}$ hours after the race.

URINE RESULTS. (In average form unless otherwise stated.)

Specific gravity. The night and morning specimens averaged 1025, the "after exercise" specimen was 1021, while after the race it was 1019 and $1\frac{1}{2}$ hours later it was 1017.

Protein. (Trichloroacetic acid.) One specimen showed a slight trace at night, four showed a slight trace after exercise and one a definite cloud, whereas after the race only one was free and he was unable to produce a specimen until $1\frac{1}{2}$ hours after the race. The others all gave a definite cloud in one or both specimens.

Reducing substances. (Benedict and Fehling.) These were only found once in the forty specimens collected and then there was only a slight trace.

QUANTITATIVE ESTIMATIONS are given in Table I as averages since all the figures varied in the same direction.

TABLE I.

	Total solids per litre	Urea		g. total N ₂ /100 g. solid	Creatinine	
		g./100 g. solid	p.c. total N ₂		g./100 g. solid	p.c. total N ₂
Night, Mar. 30th	65.31	53.08	98.02	25.27	2.02	2.9
Morning, Mar. 31st	65.93	58.00	97.53	27.75	1.16	1.5
After exercise, 31st	55.64	49.20	94.92	24.19	4.55	6.9
After race, April 1st	49.08	45.83	93.90	22.78	3.68	6.0
$1\frac{1}{2}$ hours after race	43.92	46.70	94.30	23.11	4.71	7.5

BLOOD RESULTS.

Blood was collected as soon as possible after the race from three volunteers, Nos. 3, 5 and 7 in the boat. The actual times of collection and the results are shown in Table II.

TABLE II.

No. in boat	Minutes after race ended	Lactic acid mg./100 c.c.	Glucose mg./100 c.c.	Non-protein N ₂ mg./100 c.c.
5	13	76	161	54
3	17	43	153	32
7	22	43	191	47

These analyses were made possible by the courtesy of the President of the C.U.B.C., Mr C. J. S. Sergel, to whom we wish to tender our sincere thanks.

PROCEEDINGS
OF THE
PHYSIOLOGICAL SOCIETY,
June 10, 1933.

Azo-dyes as anti-coagulants. By A. ST G. HUGGETT and F. M. ROWE.
(*Departments of Physiology and of Dyeing and Colour Chemistry, University of Leeds.*)

In 1930, Rous, Gilding and Smith showed that Chicago Blue 6 B prevented blood clotting. In 1932, Huggett and Silman showed that Chlorazol Sky Blue FF (an English equivalent of Chicago Blue 6 B) stopped coagulation by acting as an anti-thrombokinase. Since then it has come into general use as an anti-coagulant. Its disadvantage is that it renders the blood a uniform ink colour. We therefore have investigated a number of azo-dyes and other substances to obtain one which was equally or more efficient and which did not discolour the blood.

Anti-coagulation we have found to be a property shared by many azo-dyes. It appears to be dependent on the chemical structure of the dye and not on the molecular weight. It is easily modified by the impurities which are present in all commercial dyes, notably heavy metal salts and sodium chloride, the latter occurring in concentrations as high as 70 p.c. Dr F. L. Pyman, F.R.S., of Messrs Boots Pure Drug Co., has kindly purified for us a number of commercial dyes and given us analyses for the impurities present.

We find that Chlorazol Fast Pink BKS (Colour Index No. 353) is more efficient than either heparine or any azo-dye which we have tested by *in vitro* tests. On injection it is not appreciably toxic, but needs to be injected in concentrations of 150–160 mg. per kg. of body weight to produce prolonged increase in the clotting time. This is due to the high solubility and diffusibility of the dye which leads to its fairly rapid excretion. Messrs The Imperial Chemical Industries Ltd. (Dyestuffs Group) kindly prepared an isomer which is less soluble and less diffusible: in the same dosage this kept the clotting time of circulating blood above normal for a longer time. This drug, S.D. 2, is the best we have obtained. Unfortunately, its decreased solubility in alkaline solutions leads to greater toxicity. The subjoined table shows the relative efficiency of heparine and certain azo-dyes.

Active and minimal lethal doses of azo-dyes.

	Wt. in mg. prolonging clotting time to 20 min. <i>in vitro</i>		Duration over which clotting time is increased above normal by intravenous injection of 160 mg./kg. body weight hr.	Minimal lethal dose on intravenous injection of the drug in rabbits mg.
	mg./c.c. blood	mg./kg. body wt.		
Chicago Blue 6 B ...	0.9	70	12	350
Chlorazol Blue FF ...	1.25	100	12	300
Chlorazol Fast Pink BKS	0.20	16	10	300
S.D. 2	0.5	40	15	160
Heparin	0.25	20	5	300

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The effect of the removal of the parathyroid glands in dogs upon the gastric motility and secretions. By J. A. LENNOX (introduced by D. BURNS).

A standard meal of meat extract was used throughout as it was found to be most readily accepted and to give a satisfactory acid and pepsin response. Samples were withdrawn every half-hour up to 2 hours by means of a stomach tube and syringe. Each dog was trained to accept the passing of the tube without any resistance or fear, and after a few weeks accepted the procedure as a normal occurrence.

Complete thyroparathyroidectomy was carried out in each case, no attempt being made to remove the parathyroid glands and leave the thyroid intact. The tetany symptoms generally became evident between the 3rd and 5th day after operation. A series of meals was given before the removal of the glands, and the acid and pepsin response standardized for the individual dog. In order to exclude the effects of the operation upon the secretions, a meal was carried out the day after the operation before any signs of tetany were apparent. Upon the tetany becoming evident, the dog was given a meal and a further series of gastric samples obtained. The meat extract accentuated and hastened the severity of the tetany, and generally, before the final sample was obtained, the dog was in severe tetany.

A marked fall in the free and combined acidity of the gastric juice was found to take place following the removal of the parathyroid glands. This was accompanied by a very definite fall in the activity of the juice, as estimated by the pepsin content. These changes in the pepsin content may be the earliest signs of latent or approaching tetany, and have been found to be present before any other symptoms were observed. That alteration in the acidity and the activity of the gastric juice is not due to the moribund condition of the animal is shown by the fact that the acid and pepsin curves rise towards the end and, in one case where the dog died immediately following the 2-hour sample, the secretions were approaching normal. The graphs of the acidity and pepsin content suggest that there is a delay in the response rather than a failure of the secretions following parathyroidectomy.

Calcium was administered during active tetany and the effect upon gastric secretion observed. Within 30 min. of the subcutaneous or intraperitoneal injection of calcium, there is a rapid return of the gastric secretions to normal. This coincides with the passing of the active tetany and the approach of the depressed state.

A series of observations upon the effect of the removal of the parathyroid glands upon gastric motility was carried out; both by means of the opaque meal and by means of opaque sutures outlining the curvatures of the stomach (method of McSwiney). These showed that there was a tendency towards depression of the motility. In one case rapid emptying of the stomach was observed immediately preceding death. This, however, may have been a purely terminal phenomenon due to the lack of tone of the pylorus.

A typical example of the results obtained.

Dog No. 4. About 6 months old.

Specimen	Total acidity of gastric juice, p.c.		
	Before operation	During tetany	After calcium
(1) $\frac{1}{2}$ hr.	0.1606	0.0585	0.146
(2) 1 hr.	0.292	0.0803	0.2628
(3) $1\frac{1}{2}$ hr.	0.328	0.0940	0.3066
(4) 2 hr.	0.292	0.1104	0.292

Pepsin estimation¹.

Specimen	Before operation	During tetany	After calcium
(1) $\frac{1}{2}$ hr.	2	—	2
(2) 1 hr.	3	1	3
(3) $1\frac{1}{2}$ hr.	4	2	4
(4) 2 hr.	4	2	4

¹ These figures are comparative and are arrived at by taking the amount of protein digested by various dilutions of the gastric juice under constant conditions.

The emptying time of the stomach for hypotonic meals.

By B. A. McSWINEY and W. R. SPURRELL. (*Department of Physiology, University of Leeds.*)

In a previous communication we gave the results obtained by the "outline method" on the rate of gastric emptying of hypertonic meals. In agreement with other observers, it was found that delay, proportional to the degree of hypertonicity, was produced.

We have performed similar experiments with hypotonic meals. The standard meal of skim milk was dialysed for 24 hours against three changes of distilled water, the depression of the freezing-point being reduced to 0.035° . The emptying time for 250 c.c. skim milk was first determined by the outline method: the effect of hypotonic solutions was then observed by feeding the same animals with 250 c.c. of the dialysed mixture. Three meals of the hypotonic solution were given to Dog G 9, and two meals to Dog G 10. In every case there was marked acceleration in emptying—skim milk left the stomach in $3\frac{1}{2}$ hours, and dialysed milk in $1\frac{1}{2}$ hours.

These findings are in agreement with those of Macleod and others [1930] for the rat, but differ from those of Carnot and Chassevant [1905] and Otto [1905] in the dog. The latter observers employed duodenal fistulae to measure the rate of pyloric outflow, and it is suggested that the functional division of the gastro-intestinal tract might be responsible for this discrepancy in results.

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The measurement of the hydrogen ion concentration of blood by the glass electrode. By S. DICKINSON, R. E. HAVARD and B. S. PLATT. (*School of Medicine, University of Leeds.*)

Two glass electrodes have been set up and used for the determination of the pH of blood. The first makes use of the form of the glass electrode described by Stadie, O'Brien and Laug [1931] and the Lindemann electrometer for the measurement of potential differences [Kerridge, 1926]. The second uses the original Haber bulb form of the glass electrode and the electrometer valve for the measurement of potential differences

[Platt and Winfield, 1933]. It will be described in detail in a separate communication [Dickinson and Platt, 1933]. Readings can be made with the first instrument to 0.2 mv.; with the second to 0.1 mv.

Both instruments have on one side of the membrane $N/10$ HCl, and make use of $N/10$ HCl quinhydrone electrode as half-cells. They are standardized by measuring the potentials given by phosphate buffer solutions of known pH in the neighbourhood of 7.4 [Hastings and Sendroy, 1924]. This is replaced by normal saline, which is in turn replaced by the sample of blood under investigation.

Temperature control is obtained in the Stadie form by a water bath surrounding the cell containing blood. In the second instrument it is obtained by means of an air bath. All experiments have been done at body temperature.

Twenty-six samples of freshly drawn human blood (including both venous and arterial) have been investigated, six with the first instrument, twenty with the second. In no case was an acid change observed which corresponded to that reported by Havard and Kerridge [1929].

The following calculation of changes of potential with changes of temperature may throw light upon this observation.

The potential difference (E) between the two sides of the membrane is given by the equation:

$$E = A (pH_1 - pH_2), \quad \dots\dots(1)$$

where pH_1 and pH_2 refer to the solutions on each side of the membrane, and A is a coefficient proportional to the absolute temperature.

The temperature coefficient of the pH of blood has been given as 0.02 pH per degree; $pH = 7.40$ is a representative value for blood at $37^\circ C$. The value of A at $37^\circ C$. is 61.5, and at $36^\circ C$., 61.3.

If these values are substituted in equation (1), the potential difference at $37^\circ C$. (E_{37}) and at $36^\circ C$. (E_{36}) can be obtained:

1. With $N/10$ HCl (pH 1.08) on one side of the membrane:

$$(a) E_{37} = 61.5 (7.40 - 1.08) = 388.7 \text{ mv.},$$

$$(b) E_{36} = 61.3 (7.42 - 1.08) = 388.6 \text{ mv.},$$

i.e. a change of 0.1 mv. per degree Centigrade.

2. With phosphate buffer of pH 7.00 on one side of the membrane:

$$(a) E_{37} = 61.5 (7.40 - 7.00) = 24.6 \text{ mv.},$$

$$(b) E_{36} = 61.3 (7.42 - 7.00) = 25.7 \text{ mv.},$$

i.e. a change of 1.1 mv. per degree Centigrade.

If the pH is calculated from the potential difference on the assumption that the temperature is unchanged at $37^\circ C$., a warming up of $1^\circ C$. will

cause, in case 1 (which corresponds to the arrangement used by us), an apparent alkaline change of 0.002 pH; in case 2 (which corresponds to the arrangement used by Havard and Kerridge), an apparent acid change of 0.018 pH. A warming up process after the blood has been put in may be the explanation of the "acid change," observed by Havard and Kerridge but not confirmed by ourselves.

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Reflex interdependence of the internal and external anal sphincters. By R. C. GARRY. (*Institute of Physiology, Glasgow University.*)

Spinal anaesthesia in the cat causes marked relaxation of the anal canal. After division of the pudendal nerves, spinal anaesthesia has little effect on the already relaxed anal canal.

It is natural to deduce that the contraction of the anal canal is largely maintained by the external anal sphincter innervated by the pudendal nerves.

However, division of the spinal rami to the inferior mesenteric ganglia causes very marked relaxation of the anal canal in the face of intact pudendal nerves. After division of the pudendal nerves section of the spinal rami to the inferior mesenteric ganglia is without effect. If it be true that the major portion of the constriction of the anal canal is maintained by impulses in the pudendal nerves, why should division of the spinal rami bring about such complete relaxation of the anal canal in spite of the integrity of the pudendal nerves? It may be argued that afferent impulses in the spinal rami reflexly maintain tone in the external anal sphincter. On the other hand, the fact that division of the pudendal nerves diminishes inhibition of the distal part of the colon [Garry, 1933] suggests that afferent impulses in the pudendal nerves not only augment the inhibitor action of the lumbar outflow on the colon, but also maintain the motor action of the lumbar outflow on the internal anal sphincter.

That such impulses in the pudendal nerves arise in the tonically contracted external anal sphincter is suggested by the work of Matti [1909]. Matti found that even very imperfect surgical restoration of

a ruptured external anal sphincter completely cured rectal incontinence in man. Matti was unable to believe that the degree of restoration of function could be explained mechanically. Experimental work on dogs confirmed these observations on man. Preservation of a relatively small portion of the external anal sphincter was able to maintain practically normal closure of the anal canal.

Matti concludes that the presence of even a few fibres of the external sphincter stimulates the underlying smooth sphincter to contraction and goes out of his way to scout the possibility of a reflex influence of the external on the internal sphincter. Significantly, however, Matti found that removal of the tonic contraction of the striped sphincter by bilateral section of the muscle bellies not only caused relaxation of the anal canal but also increased the activity of the colon and the ease with which defæcation could be elicited.

Such findings seem to be best explained by the hypothesis put forward from work on the cat.

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The effects of certain tissue extracts on pregnancy. By J. M. ROBSON¹. (*Institute of Animal Genetics, University of Edinburgh.*)

The subcutaneous injection of certain saline extracts into pregnant mice produces interruption of pregnancy. Hourly injections over a period of 8 hours have been given. Three types of effects have been obtained, namely:

- (1) Abortion of apparently normal foetuses with placentæ and membranes within 12 hours of the injections.
- (2) Delayed abortion of dead or macerated foetuses, and
- (3) Resorption.

Active extracts have been obtained from placental tissue, foetal tissue and liver. All effects have also been obtained with protein free extracts, and moreover the active substance appears to be soluble in ether (aqueous). Histamine in doses up to 2 mg. does not produce the above effects. The activity of extracts rapidly decreases and marked differences between the potency of extracts prepared under apparently the same conditions have been observed. The effects are not due to œstrin or gonadotropic hormone.

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Depressor shock. By R. J. S. McDOWALL.

Some cats under dial or chloralose anaesthesia are extremely sensitive to depressor effects of mechanical or slow galvanic stimuli. In many instances a shock-like condition is produced by operative processes which would otherwise be quite innocuous. They occur under artificial respiration with the chest freely open but may be prevented by deep anaesthesia and by ergotamine and may be counteracted temporarily by faradic stimulation of the sensory nerve. The exact conditions of the experiment have not yet been made out but there is evidence of seasonal differences, the results being unobtainable from October to December.

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